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Modelling DNA in aqueous solutions

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Summary — A surge in theoretical and computer simulation studies on DNA has been witnessed during the last few years. These are focused on addressing problems concerning the molecular basis of conformational transitions of DNA, base sequence specific structural modulations (fine structure) of DNA and structural and thermodynamic aspects of recognition in protein-DNA and drug-DNA systems. Computer modelling of DNA is now becoming increasingly popular in the research and developmental activity in pharmaceutical industry. Several models of DNA have been put forth to describe DNA. These range from very simple descriptions, such as a line of charge, uniformly charged cylinder, to more complex models of DNA, with water and supporting electrolyte considered explicitly at an atomic level. The techniques employed include the more common molecular mechanics and quantum and statistical mechanical methods. This article presents the progress attained via the diverse theoretical and computer simulation studies on DNA with a special emphasis on the methodological issues involved in modelling DNA in aqueous solutions.

Introduction

Since the epoch-making first molecular model of DNA was proposed four decades ago[1,2], there has been a tremendous growth in our knowledge of the molecular biology of the gene[3]. While the scientific world waited for at least a score and six years more to visualize the structure of DNA at atomic resolution through progresses in single crystal studies on DNA[4], both theory and experiment benefited significantly from the atomic coordinates of the canonical B-DNA reported in early seventies[5] based on fibre diffraction data. This coupled with the availability of ever increasing computing power and advances made in adapting numerical techniques to biomolecular problems has led to a surge in theoretical explorations of DNA structure and function. The aim of most of these theoretical efforts, more generally, has been to arrive at an accurate description of DNA in aqueous solutions, to present a molecular perspective on conformational transitions of DNA, sequence specific structural modulations (fine structure) of DNA and to understand the molecular basis of protein-DNA and drug-DNA interactions. From *in vacuo* models of DNA described at the atomic level, the focus of current research is shifting to *in vitro (in aquo)* models and this will hopefully pave the way for an *in vivo* model. This article presents an overview of some of the recent applications of different theoretical and computational studies aimed at enhancing our understanding of the nucleic acid systems, with special emphasis on modelling DNA in aqueous solutions.

DNA at physiological *p*H occurs as a polyanion, the acidic phosphate groups being fully ionized[6]. An equivalent number of counterions (Na^+, K^+ or Mg^{2+}, etc.) present in the system, maintain electroneutrality. This together with solvent water and added salt (e.g. NaCl, KCl or $MgCl_2$) constitutes a typical DNA system *in vitro* or *in vivo*. Both, the base sequence of DNA and its environment (ion atmosphere and solvent), are expected to be the major determinants of the structure and function of nucleic acids. Theoretical studies of nucleic acids can be classified into two distinct areas. One area has focused almost entirely on electrostatic properties such as the potentials and ion atmosphere that surround the macromolecule using simplified models of DNA—a line of charge or a charged cylinder—but taking cognizance of its environment. The work of Fuoss *et al.*[7] and Alfrey *et al.*[8] was along these lines. Hill[9]

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modelled DNA as a uniformly charged cylinder as early as 1955, keeping in view the Watson-Crick (WC) structure. Such models are in vogue even to this day for an estimation of the macroscopic properties (e.g. colligative properties) of DNA in solution. The other area of theoretical endeavour has concentrated on the details of DNA conformation and dynamics working with atomic models, for example, as in quantum or molecular mechanics calculations or molecular dynamics simulations, but has tended to either neglect solvent and ionic strength effects, or treat them in an *ad hoc* fashion. A description of the atomic model of DNA along with an explicit consideration of the solvent and supporting electrolyte medium has become feasible only recently[10].

The diverse theoretical and computational studies leading to an appreciation of the structure, dynamics and thermodynamics of DNA systems are considered in this article—viewed as a hierarchy of theoretical models—alongside the related issues of structure and energetics of the counterion atmosphere of DNA and solvent organization. Counterion condensation, a crucial concept in understanding the behaviour of nucleic acids in aqueous systems, is introduced in the following section. Poisson-Boltzmann studies which have become popular in recent years are reviewed in the next section emphasizing applications to aqueous solutions of DNA. The pioneering work of Pullman and coworkers on molecular electrostatic potentials of DNA systems, based on quantum mechanics, is briefly

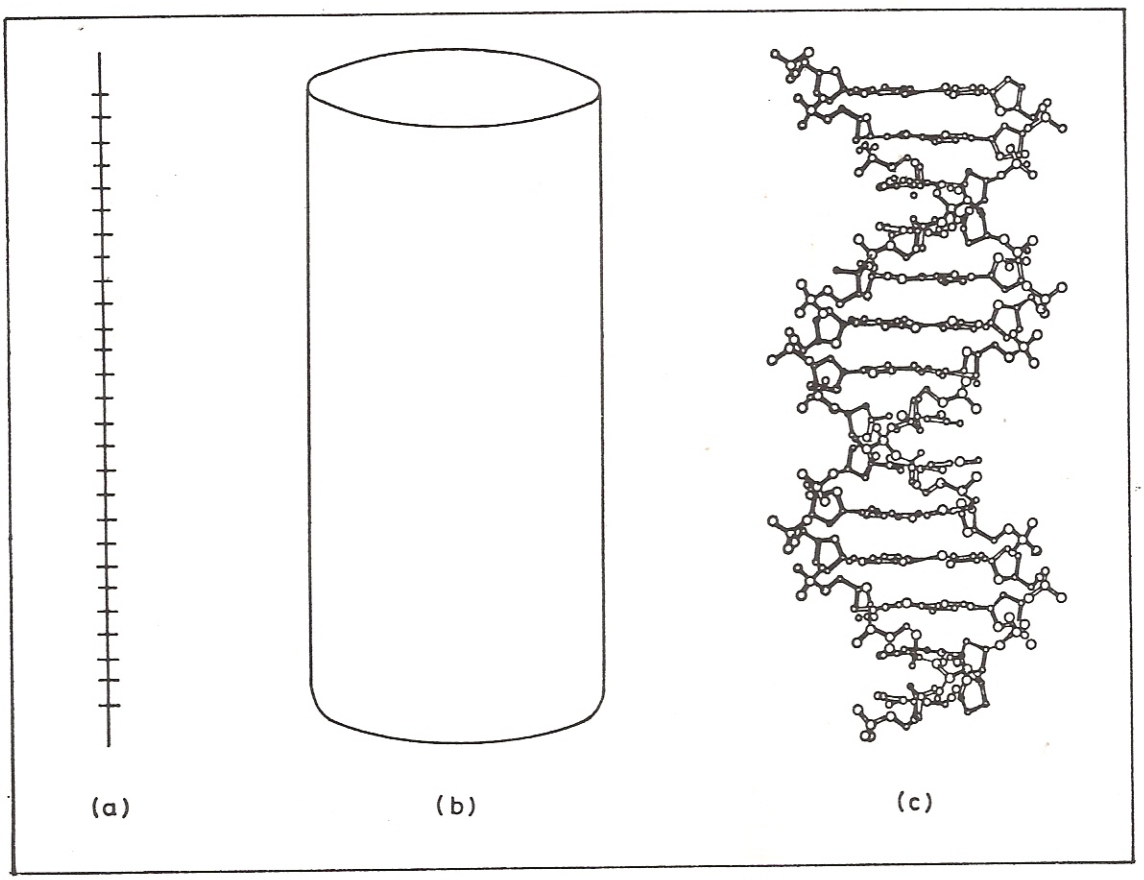

Fig. 1

(a) — B-DNA described as a linear lattice with negative charges located at a distance of 1.7Å from each other. This is the underlying model in the remarkably successful counterion condensation theory.

(b) — B-DNA described as a uniformly charged cylinder. Its potential varies as log(distance) and it displays excluded volume. This model mimics DNA at larger distances.

(c) — All atom model of B-DNA with atomic coordinates obtained from X-ray or fibre diffraction data. This model is empolyed extensively, with or without solvent and added salt (supporting electrolyte), in molecular mechanics calculations, Monte Carlo and molecular dynamics simulations, finite difference Poisson- Boltzmann studies, etc.

described. Statistical mechanical techniques and computer simulations as applied to DNA are summarized in the subsequent sections. Molecular mechanics and dynamics studies on DNA without solvent and counterions are too numerous to be quoted here. Similarly, novel DNA structures deserve a special treatment elsewhere. For a larger purview on the subject the reader may refer to the more comprehensive and specialized reviews on condensation theory[11,12], ion atmosphere of DNA[13-17], molecular electrostatic potentials[18,19], hydration[20,21], molecular mechanics and dynamics of DNA[22-24], DNA supercoiling[25,26] and modelling drug-DNA interactions[27]. A perspective, at times subjective, as discernible from the various theoretical and computational studies addressed in this article, is presented on the current status of knowledge on DNA systems *in vitro*.

Counterion Condensation in Nucleic Acid Systems

Counterion atmosphere of DNA neutralizes the charges of the anionic phosphates and imparts electrostatic stability to the system. The nature of the ion atmosphere of DNA has therefore been the subject of considerable research attention, both experimental and theoretical, in recent years. An important organizing principle is the phenomenon of 'counterion condensation' (CC) (Refs 12, 28, 29); no matter how dilute the solution, a number of the counterions remain in close proximity to the DNA, compensating a large percentage of the phosphate charges. These counterions, some fraction of the total, are said to be "condensed". The remaining fraction of counterions is considered to form a Debye-Huckel type diffuse ionic cloud. An entropy of mixing term completes the picture. Minimization of the phenomenological free energy expression for DNA treated as an infinite line of charge in the limit of infinite dilution resulted in net phosphate charges screened from –1 to –0.24 by the condensed monovalent counterions.

$$\xi = q^2/\varepsilon kTb;$$

$$\text{Magnitude of the net charge} = (1/N\xi) \quad \ldots (1)$$

where b is the average axial charge spacing taken as 1.7 Å for the canonical B form of DNA; ξ is a dimensionless structural parameter defined as proportional to the charge density and for water at 25°C, ξ=7.1/ b (in Å) and is equal to 4.2 for B-DNA and N is the valency of the counterion. Thus the net charge/phosphate in aqueous solutions of B-DNA is –0.24 with sodium counterions and –0.12 with magnesium counterions.

According to an observation attributed to Onsager[30], statistical mechanical phase integral for an infinite line of charge model diverges for all values of linear charge density (ξ) greater than a critical value (unity). This divergence implies instability. A sufficient number of counterions will condense to reduce (ξ) to a value less than unity. The idea of counterion condensation follows simply from thermodynamic arguments[12]. Small ion–pairing is well known to decrease with dilution due to the increased potential for a large entropy of mixing, which favours dissociation. For polyions like DNA, the superposition of the electrostatic potentials of the phosphate groups on any given mobile counterion makes enthalpic effects dominant in the equilibrium, and as a consequence a significant fraction of counterions remain associated with the DNA, regardless of the bulk salt concentration. Thus, counterion condensation is unique in polyelectrolytes compared to simple electrolyte systems[12,29]. As opposed to site binding, condensation according to Manning, implies that all condensed counterions are in a state of complete hydration and free translational and rotational mobility, i.e. delocalized and rather loosely associated with the DNA. The Manning radius (i.e. the radius of the coaxial cylinder around DNA enclosing 76% of net counterionic charge per phosphate) of the counterion condensate is typically ~7 Å (ref.12) beyond the surface of the DNA (and ~17Å from the helix axis). Counterion condensation and displacement are collectively known as polyelectrolyte effects and play an important role in many aspects of the structure and function of nucleic acids. Release of condensed counterions is considered to be an important thermodynamic component of ligand and protein binding to DNA[12,15]. Manning has provided an account of diverse properties of DNA (colligative properties, transport properties, binding equilibria, melting temperatures, etc.) via counterion condensation theory[11,12,31,32] and the agreement between experiment and CC theory is considered good. Dewey[33] developed a counterion condensation model applicable to oligoelectrolytes at high ionic strength. Several ^{23}Na NMR experiments and related studies[34,45] addressing the role of ion atmosphere support the predictions of the CC theory.

Recently, Fenley *et al.*[46] adapted the counterion condensation theory to a more realistic representation of DNA as a three dimensional discrete charge distribution. They found the results to conform to the inferences based on experiment and the simple linear lattice model with a uniform dielectric constant[12], when dielectric saturation function was considered by means of a distance dependent dielectric function for the interactions

between phosphates on a double helical array in a B-DNA geometry. The simpler the DNA model, the simpler the solvent description, or if stated alternatively, a more realistic solute model necessitates a more complicated solvent description! In a subsequent numerical analysis[47] of the CC theory of the B to Z DNA[48,49] transition, they observed that the extended counterion condensation theory captures the essentials of the B to Z DNA phase diagram constructed as a function of ionic strength. The need for a better understanding of the short-range solvent mediated forces was underscored in this study for a quantitative assessment of the free energy changes in conformational transitions.

Poisson-Boltzmann Studies

A well known theoretical approach for determining the electrostatic potentials around macromolecules is based on solutions to the Poisson-Boltzmann (PB) equation (Eq.2)

$$\nabla \{\varepsilon(x) \nabla \phi(x) - \bar{\kappa}^2(x) \sinh \{\phi(x)\} + 4\pi\rho_f(x) = 0 \quad \ldots(2)$$

where ϕ is the potential in units of (kT/e), ε is the dielectric function, $\bar{\kappa}$ is the modified Debye-Huckel parameter ($\bar{\kappa} = \varepsilon^{1/2}\kappa$) where $1/\kappa$ is the Debye length and ρ_f is the fixed charge density. The electrostatic potential and the counterion concentration are inter-related via the Boltzmann equation: $\rho_+ = 2C_0 \sinh \{\phi(x)\}$; where C_0 is the bulk salt concentration. The PB equation can be solved analytically for simple geometries such as a line of charge and a uniformly charged cylinder at zero added salt in a dielectric continuum solvent characterized by a uniform dielectric constant ε. Numerical solutions can be sought in other cases. Solutions to the PB equation yield potentials, fields, electrostatic free energies and small ion (counter and coion) concentrations.

The earliest studies of this genre were conducted on simplified models of DNA[50-58]. Zimm and Le Bret[55] showed elegantly using the PB equation how a rodlike polyanion, like DNA, on increasing dilution condenses naturally the counterions at a level intermediate between a charged sheet (100% condensation, the Gouy-Chapman double layer) and a charged sphere (~0%). Anderson *et al.*[14,16,56], have investigated numerous aspects of the PB treatment of ion atmosphere vis-a-vis thermodynamic measurements and NMR spectroscopy, with a focus on the salt dependence of the fraction of the condensed counterions. In these and other calculations[59-64], limitations of the PB theory due to neglect of finite size of the mobile ions and spatial correlations have been characterized by comparisons with Monte Carlo (MC) simulations and Hypernetted Chain (HNC) theory[65]. Qualitative agreement among the various theoretical methods has been attained. The PB calculations have been used to investigate Manning's CC theory prediction about the insensitivity of charge compensation of phosphates to added salt concentration. It showed slight but potentially significant variation [60,61]. A recent analysis of the solutions of cylindrical PB equation, as applied to DNA in aqueous solutions[66], suggests that there exist certain spatial scales other than "Manning radius" which are invariant to added salt. Implications of this observation are yet to be grasped.

Theoretical studies of electrostatic interactions at finite salt concentrations, have been extended to treat all-atom models of DNA[67-69]. Klein and Pack[67,68] obtained electrostatic potentials from an iterative PB solution to a combination of Coulombic potentials from the fixed macromolecular charges and the distribution of mobile charges, obtained from the Boltzmann equation. The results predict significant concentration of mobile cations in the minor groove as well as along the sugar phosphate backbone, a consequence of the superposition of transgroove anionic phosphate potentials. Calculations of the electrostatic interactions of the B and Z conformers of DNA with an environment of 0.01*M* monovalent salt showed that the salt exerted a greater stabilizing effect on the left handed Z-conformer than on the right handed B-form. However, this treatment assumes that the dielectric constant ε was 80 everywhere, including inside the DNA.

The potential influence of the dielectric boundary between the DNA (ε = 2-4 ?) and solvent at $\varepsilon \sim 80$ was explored recently by several groups[70-73]. The SATK (static accessibility Tanford-Kirkwood) model of Matthew and Richards[70] predicted the highest concentrations of the mobile cations in the minor groove of DNA, in agreement with Klein and Pack[67,68] and provides a better agreement with ion distribution data obtained by charge transfer experiments[71] than is done by PB solutions for a cylindrical uniform dielectric model. The calculated electrostatic free energies of A, B and Z forms of DNA showed that Z-DNA is the most stable form at high ionic strength. While this and related work illustrates the importance of dielectric boundary effects, a spherical model, as in SATK studies, is inherently limited when describing the detailed shape of a complex molecule such as DNA. Extensions of the Tanford-Kirkwood model to arbitrary

charge distributions with an overall cylindrical symmetry have since been reported[58] and it is now feasible to carry out SATK type calculations on DNA. Troll *et al.*[72] conducted a macroscopic simulation of duplex DNA represented by a clay model in an electrolyte tank. It was found that (a) interactions between charges on the same side of the DNA were enhanced as a consequence of concentration of field lines by the low dielectric DNA, increasing both attractive phosphate-cation and repulsive phosphate-phosphate and cation-cation interactions, and (b) shielding is increased for charges on opposite sides of DNA by the presence of the low dielectric medium. The net effect of the dielectric boundary on the system is a diminished tendency for the residency of cations in the grooves of the double helix.

Jayaram *et al.*[73] have incorporated dielectric boundary, solvent screening and ionic strength into finite difference solutions to the PB equations (FDPB[74,75]) for an all-atom model of DNA. Sequence dependence in electrostatic potentials appears in this study as a natural consequence of the model with no additional assumptions. The numerical results qualitatively support the electrolyte tank observations[72] on the angle dependence of the charge-charge interactions. In contrast to the tank results, FDPB calculations show an overall increase in the groove populations of counterions at low ionic strengths. The calculated electrostatic contribution to the total interaction energy of an AT base pair turned out to be about–4.6 kcal/mol and that for a GC base pair–6.4 kcal/mol (Jayaram and Honig, unpublished results; Nidhi Aneja and Jayaram, work in progress) which is very close to the experimental value of –2 kcal/H bond of Turner *et al.*[76]. Electrostatics seems to dominate the base pairing interactions. Friedman and Honig[77], however, showed that electrostatics contribute negligibly to stacking interactions. Recently, the FDPB method was used to analyze drug-DNA (minor groove binders, Vandana Arora and Jayaram, unpublished work, 1992) and protein-DNA (λ repressor-operator, Achintya Das and Jayaram, unpublished work, 1992) interactions. While qualitative features of complementarity were established, it was concluded that focussing, rotational averaging[75] and other error minimizing techniques had to be employed for estimating the electrostatic contribution to the free energy of binding. A detailed analysis of electrostatic effects in protein-DNA interactions using a combination of FDPB method and molecular dynamics is underway [Jayaram, McConnell and Beveridge, work in progress].

The FDPB approach provides a conceptually simple and a theoretically sound basis to investigate electrostatic interactions involving nucleic acids (biomolecules in general), under physiological conditions[78]. No *ad hoc* hypotheses, such as charge neutralization of phosphates, are invoked to obtain 'physically resonable' numerical estimates of electrostatic potentials around DNA and to understand base specificities. The electrostatic potentials obtained with this approach can, in principle, be incorporated either directly or as a look-up table of effective dielectric constants[73] into the potential functions in the context of energy minimization studies and in Monte Carlo/molecular dynamics simulations and may be periodically updated during the course of the simulation. Gilson *et al.*[79] have recently described a method to compute electrostatic forces accurately using the Poisson-Boltzmann equation which can be integrated into molecular mechanics and dynamics protocols. You and Harvey[80] described an implementation of the finite element approach to the evaluation of the electrostatics of macromolecules with arbitrary geometries. Extension of this work to DNA is awaited.

Solvent water, in all the studies enumerated above, is treated as if the dielectric constant was everywhere equal to the bulk value of ~80. It is feasible in principle in FDPB calculations on DNA to incorporate an extra layer of solvent around the solute with a lower dielectric constant (ε = 20?) than the bulk but such parameters are hard to calibrate and justify. Clearly, further studies are required, both experimental and theoretical, on the microscopic dielectric behaviour of the environment around DNA. This, notwithstanding, role of dielectric inhomogeneity and the influence of dielectric saturation[81] on oligonucleotide-small ion interactions has been studied by Hingerty *et al.*[82] by means of a distance dependent dielectric screening function, subsequently employed in energy minimization studies on DNA by Lavery and coworkers using the JUMNA method[83]. The dielectric function employed in these studies has a sigmoidal form (Eq.3).

$$\varepsilon(r) = \varepsilon - [\{(\varepsilon - 1)/2\} \{ (rs)^2 + 2rs + 2\} \exp(-rs)];$$
$$\varepsilon = 78 \text{ and } s = 0.16 \qquad \ldots(3)$$

Mazur and Jernigan[84] have recently reviewed the applicability of distance-dependent dielectric constants and have suggested some modifications. Their analysis indicates that the local helix geometry of the base pairs is strongly affected by the contributions of the electrostatic interactions to the stacking energy. Over all, support for the inclusion of dielectric saturation in dealing with charge-charge interactions in DNA systems in

the dielectric continuum solvent approach appears to be mounting.

An issue of considerable theoretical interest arising out of the application of nonlinear Poisson-Boltzmann equation to DNA at physiological ionic strengths, has been the evaluation of energetics avoiding inconsistencies. One way out is to use the linearized PB equation along with the condensation theory postulate[46,58]. Gueron and Demaret[85] developed an algebraic approximation for calculating the Poisson-Boltzmann free energy of a cylinder and provided a simple explanation of the electrostatics of the B to Z transition of DNA[86]. Sharp and Honig[87] formulated the problem in terms of an energy density integral and laid out a framework in which it was feasible to obtain the electrostatic free energies in a consistent fashion. In particular, they found that the energy density integral includes excess osmotic pressure of the ion atmosphere, a term which is often overlooked. This allows a rigorous numerical treatment of energetics of solvation, conformational equilibria and ligand binding involving DNA at finite salt concentrations, *albeit* at the level of continuum solvent and ion atmosphere.

Quantum Mechanical Studies

Some of the earliest calculations on electrostatic potentials around nuleic acids including detailed descriptions of the geometry of DNA were carried out by Pullman and coworkers[18,88]. The electronic wave functions of the constituent units of the nucleic acids were first obtained by *ab initio* self- consistent field calculations. The electrostatic molecular potential of each constituent unit was then evaluated using the electron-density distribution (Eq.4)

$$\Phi(P) = \sum_{\alpha} \frac{Z_\alpha}{r_{\alpha P}} - \int \frac{\rho(i)}{r_{Pi}} d\tau_i \qquad \ldots (4)$$

where ϕ (P) is the value of the potential at a given point P in space, Z_α are the nuclear charges and ρ is the electron density distribution, $r_{\alpha p}$ and r_{pi} are the respective distances between point P and nucleus α, and point P and electronic charge element $\rho(i)d\tau(i)$ in volume element $d\tau_i$.

The potentials of more complex units such as DNA were constructed using an overlap multipole expansion procedure.

These studies formed the basis for a qualitative interpretation of several succeeding experimental and theoretical studies. Two major points of interest in these studies were: (i) deepest potentials were located in the grooves as opposed to the phosphate regions, and (ii) electrostatic potentials in conjunction with solvent accessibilities of the surface atoms enabled formulation of a reactivity scale for electrophilic attack. Based on these studies, it was concluded that for B-DNA, N7 and O6 of guanine in the major groove should constitute the most significant attractive centers of a GC base pair for electrophilic reactants, and N3 of adenine and O2 of thymine in the minor groove should constitute such centres for AT pairs. The distribution of the electrostatic potential in B-DNA shows an intrinsic dissymetry (the minor groove of AT sequences is more negative than the minor groove of GC sequences). Supportive evidence for these observations comes from the Poisson-Boltzmann calculations[73] carried out on B-DNA in a continuum solvent under physiological salt concentrations. The reactivity scales, and the potentials of course, are expected to depend upon structural assumptions and vary with the fine structure of DNA. Pullman and coworkers have also given an extensive account of the hydration of nucleic acids and their constituents via quantum mechanical calculations and these have been cited elsewhere[20,89].

Integral Equation Methods

Spatial pair correlation functions are of considerable interest in statistical mechanics in the context of structure and thermodynamics of molecular fluids[90]. One of the well known and most sought after two body spatial correlation functions, g(r), also known as the radial distribution function, is related to the structure factor S(k), obtainable from diffraction studies. g(r) is also related to the chemical potential and potential of mean force. Ornstein and Zernicke (OZ) proposed an exact integral equation relating two pair correlation functions h(r) and c(r) (ref.90)

$$h(1\,2) = c(1\,2) + \int c(1\,3)\, d(3)\, \rho\, h(3\,2) \qquad \ldots (5)$$

where h(1 2) = h(r) = {g(r)–1}; and c(1 2) = c(r) is known as the direct correlation function. A closure approximation[90], such as the mean spherical approximation (MSA). Hypernetted chain (HNC) or Percus-Yevick (PY) approximation is usually employed to solve iteratively for h(r) or g(r) and related properties. This technique is called the integral equation method and has become popular in recent years, particularly with the reformulation of the site-site OZ equation for molecular and polymeric systems.

Soumpasis[91] considered the phosphate backbone of DNA and the ion atmosphere as a fully dissociated 1:1 electrolyte of specified composition and thermodynamic state for the purpose of estimating the phosphate-phosphate potential of mean force (pmf). The pmfs were calculated using either the HNC formalism or the exponential mean spherical approximation (EXP-MSA) (ref.90) depending on the level of accuracy desired. The effective two-body potential of mean force thus obtained can be incorporated into the Hamilitonian of a many body system. This approach was shown to account for quantitatively the salt dependence of free energy differences between B and Z conformations of DNA from 0.1 to 4 *M* NaCl[92]. The theory uses only one adjustable parameter, the distance of closest approach of an anion-cation pair (set equal to 4.9Å for NaCl). The success of the theory is intriguingly remarkable considering that the derived pmfs between phosphate charges do not incorporate the effects due to the presence of other atoms on DNA nor their connectivity along the backbone of DNA. The pmf approach was subsequently integrated into AMBER force field (*see*, Refs 93-95 and references therein for other applications), replacing the electrostatic terms in it by the pmfs obtained using the integral equation methods. This enabled an investigation of the structural transitions of DNA in solution.

Recently, Hirata and Levy[96] have discussed the application of a RISM theory developed for solvated polymers to a study of the salt effects on DNA conformation. The polyion was represented by an infinite array of charged spheres equally spaced on helical chain. RISM equations for the pair correlation functions were set up for the interaction sites and solved iteratively with HNC-like closure for small ions and PY-like closure for phosphate-small ion correlations. Their studies predicted a transition from B to Z form at 3.6 *M* added salt in qualitative accord with experiment without any optimization of the model parameters involved. Their work further showed that the superposition approximation inherent in the pmf approach[91] might be less severe than anticipated (in the B to Z transition) due to the cancellation of higher order correlations when the free energy difference between B and Z-DNA was calculated.

Bacquet and Rossky[65] applied the HNC integral equation method to the system of DNA in aqueous 1:1 electrolyte solution to investigate the radial distribution of ion atmosphere around DNA. The HNC and CC are in general qualitative accord but differ in quantitative predictions regarding the extent and concentration dependence of the counterion condensation. In a related study[38] on ion distributions and competitive association in DNA/mixed salt solutions, composition near the polyion was found to be more responsive to the changes in the bulk composition. The counterion size was of minor importance compared to its valence in determining the ionic distribution, except at immediate contact with the polyion. The displacement of Na^+ by Mg^{2+} in the vicinity of polyion was approximately one for one. The calculated fluctuations in the electric field gradients experienced by the sodium nuclei correlated well with the observed NMR line widths.

The integral equation methods, accepting the approximations, are computationally more expedient than molecular simulation methods for estimating free energies. This is one area that needs to be developed to tackle biomolecular problems in general, and DNA-ligand interactions in particular.

Simulation Studies

Canonical Monte Carlo Studies

The system, in these simulations, is configured in the (T,V,N) ensemble. A choice of the DNA conformation (solute) is made and the internal degrees of freedom of the solute are frozen. The solvent and/or small ions are allowed to move probing the configuration space according to the Metropolis algorithm[97] or its variations with or without periodic boundary conditions. Several configurations ($\sim 10^6$) are generated which are consistent with the Boltzmann distribution. The resultant complexions of the system are analyzed for structural and thermodynamic indices (for further details on the methodology, *see* Refs 90 and 97-99).

Hydration

DNA presents a variable and sequence dependent hydrogen bonding pattern in the major and minor grooves[100]. Solvent water is expected to interact with the four bases— adenine, guanine, thymine and cytosine— differently and shows a differential, sequence dependent, stabilization effect on the overall conformation. With the observation of the "spine of hydration" in the minor groove of B-DNA[101], the plausible role of solvent in deciding the fine structure of DNA, the implication of water mediated hydrogen bonds in protein-DNA interactions[102] and their potential contribution to the thermodynamics of DNA-ligand binding[103], there is a renewed interest in the hydration of nucleic acids.

Early studies on hydration focused on the nucleic acid constituents[89,104]. These were subsequently extended to oligonucleotides and the dodecamer sequence[20]. Subramanian and Beveridge[105] gave a theoretical account of the sequence dependent hydration of DNA vis-a-vis X-ray[101] and calorimetric studies[103]. They noted that both AT and GC regions in the minor groove can support an extended network of water molecules owing to the geometric versatility of water-water hydrogen bonding. The penetration of water into the DNA was clearly greater for AT than for GC tracts, as anticipated. A survey of the hydration of nucleic acid systems with a nearly complete citation to the literature may be found in Ref.20. Monte Carlo simulations on water and counterions around DNA are now performed as a part of the equilibration procedure in molecular dynamics simulations on DNA in solution.

Ion Atmosphere

Monte Carlo or molecular dynamics calculations based on a fully explicit consideration of DNA, water, counterions and added salt concentration of physiological interest are a computationally expensive proposition even for current day supercomputers. Studies aimed at pursuing the role of electrostatic interactions and the nature of the ion atmosphere of DNA based on molecular simulation often take recourse to the primitive model. In all the simulation studies with the primitive model and variations thereof, ions are treated explicitly and water is represented as a dielectric continuum. This is clearly a serious approximation since the molecular nature of water as an associated liquid is neglected and its particular capacity for hydration, bonding and solvation in different modes— hydrophilic, hydrophobic and ionic— is denied. Also, there is no way to accurately parametrize the heterogeneous qualities of the dielectric mdedium from experimental data. Results of theoretical studies/simulations based on primitive model have to be interpreted in this context.

Extensive canonical Monte Carlo studies on the DNA counterion systems in the absence and presence of added salt have been reported by several groups. Le Bret and Zimm[59] modelled DNA as a linear lattice of charges imbedded in an impenetrable cylinder and also as a double helical array of charges, with mobile ions represented as hard spheres interacting with each other and with DNA via coulombic potentials in a solvent treated as a dielectric continuum. They found a striking accumulation of counterions in a layer of concentration exceeding 1 *M* at the surface of the polyion, in agreement with conclusions from previous PB studies and CC theory. Ion distributions around a uniformly charged cylinder representing DNA were subsequently explored by Murthy *et al.*[60]. The PB approach was found to underestimate counterion concentrations near DNA by about 12 to 18%. Each of the three approaches, viz. the PB, the HNC and the MC, was concluded to be internally consistent in predicting that the counterion concentrations near the polyion were essentially independent of bulk salt concentration but the empirical Manning radius decreased with increasing salt concentration. Also, the importance of the long-range interactions in systems containing DNA was emphasized.

Mills and coworkers[61-63] undertook counterion Monte Carlo calculations to investigate the role of small ion correlations in the PB theory and Manning's CC theory predictions about the insensitivity of charge compensation of phosphates to added salt concentration. The DNA in these studies is typically treated as a charged cylinder or as a lattice of discrete phosphates. The small ions were represented by hard spheres in a solvent treated as a uniform dielectric continuum. The agreement between PB and MC approaches was found to be better for larger counterions. The net positive charge in a given volume around DNA was found to vary with salt concentration in both MC and PB treatments. Conrad *et al.*[64] have incorporated effects due to dielectric discontinuity in their interaction potentials for evaluating small-ion DNA interactions. The modifications to Coulomb's law tended to drive the ions out of the grooves and especially the major groove. This they ascribed to repulsions between the ions, the low permittivity of the helix and partly due to the focusing of field lines of phosphates at the surface of the helix caused by dielectric discontinuity.

A study comprising comparative simulations of the counterion distribution around DNA based on a series of models for aqueous dielectric medium which are likely to bracket the true physical nature of the system was undertaken recently by Jayaram *et al.*[106]. The results showed that, in all cases of continuum solvent, counterion concentrations near DNA (i.e.~10 Å from the helical axis) exceeded 1*M*, even in the absence of excess salt, consistent with counterion condensation theory and previous MC and PB studies on DNA-counterion systems. An analysis of the simulation results based on different dielectric models indicated that the dielectric saturation model favoured increased counterion condensation relative to coulombic model, with DNA-counterion interactions dominating the small ion repulsions. The dielectric saturation model produced an essentially salt- independent result for the

fraction of condensed counterions over an added salt concentration range of 0 to 150 m*M*, consistent with inferences based on NMR and CC theory.

Recently, Gordon and Goldman[107] reported counterion and solvent distributions around a uniformly charged cylinder and a helical lattice as seen in their Monte Carlo calculations conducted with 15 counterions and explicit water. The smeared charge model, quite interestingly, led to a high degree of solvent polarization, causing the counterions to avoid regions vicinal to the polyion. When the helical necklace of charges at the surface of a cylinder was considered as a model for the polyion in conjunction with molecular solvent, the counterions formed a compact double layer near the surface, resulting in an extensive screening of the polyionic charges, as found earlier in the numerous studies based on continuum solvent.

Grand Canonical Monte Carlo Studies

The (T,V,μ) ensemble simulations lend a direct handle on the excess chemical potentials and excess free energies which are not as easily accessible in the canonical Monte Carlo calculations. Almost all the GCMC studies todate on DNA employ the primitive model to study the role of ion atmosphere on the bulk solution properties of DNA and no hydration studies have been reported. GCMC simulations with explicit solvent are slow to converge at particle densities of experimental interest. In the GCMC calculations, the DNA surrounded by counter and coions at a specified activity (chemical potential) and temperature is placed in a central cell. This cell is coupled to a heat bath and a particle reservoir consisting of counter and coions. Both particle displacment and insertion/deletion are carried out according to the Metropolis criterion [97]. (Details on the methodology are given in Ref.108). The GCMC simulations have provided very interesting information on non-idealities in aqueous solutions of DNA.

Vlachy and Haymet[109] employed the GCMC method to obtain structural and thermodynamic data for model polyelectrolyte solutions treating the polyion as an impenetrable, rigid, infinitely long cylinder. The results were compared with PB and HNC integral equation studies using the mean spherical approximation (MSA). The PB equation was concluded to retain its semiquantitative utility even in the range of moderate to high (1*M*) concentrations of added salt. The agreement between GCMC and HNC/MSA was found to be better, but detailed comparison with experiment was not undertaken in this study. Extensive GCMC studies on aqueous solutions of sodium salt of DNA in the presence of added simple salt were reported by Mills *et al.*[110]. The simulations were used to calculate mean ionic activity coefficients and "preferential interaction coefficients" (similar to Donnan salt exclusion factor[11] and Donnan membrane equilibrium parameter[111]) for a cell model representation of NaDNA with added salt. Results emerging from their GCMC simulations were compared with PB results and the influence of small ion correlations was analyzed and concluded to be small. In a follow-up study by Paulsen *et al.*[112], the calculated preferential interaction coefficients were compared with experimental Donnan coefficients with good agreement at low added salt. The DNA was treated as an impenetrable cylinder, with phosphate charges modelled as a continuous line of charge on the cylindrical axis in these studies.

Olmsted *et al.*[113] subsequently characterized the role of end effects on molecular and thermodynamic properties in oligoelectrolyte solutions via GCMC method. In particular, they found that the cationic concentrations at the surface of DNA oligomers were dependent upon the length of the DNA and that an oligoion containing 48 or greater number of phosphates behaved as a polyion. This study was later extended to investigate the thermodynamics of denaturation of oligonucleotides[114]. An interesting analogy between the GCMC simulations and Donnan experiments was given by Record and Richey[115]. The GCMC studies of Anderson and coworkers [112-115] on the preferential interaction coefficient which they identify as a thermodynamic measure of nonideality due to small ion-polyion interactions, have provided a considerably enhanced perspective on the problem. Valleau[116] presented an extension of the GCMC method to a flexible polyelectrolyte immersed in primitive model aqueous electrolyte solutions.

Recently, Jayaram and Beveridge[108] reported grand canonical Monte Carlo simulation studies on aqueous solutions of sodium chloride and sodium salt of DNA in the presence of added salt. Results for the simple electrolyte indicated that a soft sphere potential function for the ions in a solvent treated as a dielectric continuum, supplemented with a Gurney correction term for desolvation[117] described the behaviour of activity coefficients as a function of concentration quite well, over a concentration range 5-500 m*M*. The results on NaDNA system in the presence of added simple electrolyte provided an account of the contravariant behaviour of nonideality of mobile ions in polyelectrolyte versus simple electrolyte solutions[118]. Excess chemical potentials calculated from these

GCMC simulations[108] suggested that the counterions interact more strongly with DNA at low salt than at high salt concentrations. Record and Richey[115] have attributed this phenomenon at a molecular level to an increase in nonuniform distribution of ions around the polyion as the salt concentration is reduced. Thus nonideality of counter and coions in sodium salts of DNA in water in the presence of added sodium chloride salt increases with dilution and decreases with added salt, contrary to the behaviour in simple electrolyte solutions.

One of the limitations of the canonical and grand canonical Monte Carlo studies as described above is that the internal degrees of freedom of the solute (DNA) are frozen and it does not respond to the fluctuating environment of the solvent and ion atmosphere. One further desirable *albeit* difficult course of research to follow would be to perform an internal coordinate Monte Carlo on DNA alongside the counterion/solvent Monte Carlo. This should enable probing both the sequence, solvent and ionic strength dependent conformational flexibility of DNA. Of course, some methodological improvements, particularly on the convergence and improving acceptance ratios, are desired to facilitate this line of research.

Brownian Dynamics Simulations

Internal motions of DNA as well as the distribution and dynamics of ions around DNA in the nanosecond range can be profitably studied by Brownian dynamics simulations[119], although very few have been reported. The method essentially involves solving numerically the Langevin equation of motion (Eq. 6).

$$m\,(d\boldsymbol{\upsilon}/dt) = m\,\zeta\,\boldsymbol{\upsilon} + \mathbf{F}\,(t) + \mathbf{R}\,(t) \qquad \ldots(6)$$

where m and $\boldsymbol{\upsilon}$ are the mass and velocity of the Brownon particle. The first term on the right hand side of Eq.(6) represents frictional force, ζ is the friction coefficient of the solvent, $\mathbf{F}(t)$ represents the force due to other Brownons/species in the system, and $\mathbf{R}(t)$ is a random force. Details of the theory and methodology may be found in Refs 90 and 120, respectively.

One of such earliest calculations was reported by Barkley and Zimm[121] treating DNA as a semiflexible chain. A theoretical account of the fluorescence depolarization was developed. In a subsequent study on DNA treated as a worm like coil[122] the average fluctuations in the twist angle between bases was calculated to be about 5° and bending of the helix axis between bases ~4°. The time constants for bending and twisting were between 1 and 100 ns. The fluctuations predicted from the worm like coil model, regardless of rate, were observed to be too small to be responsible for ^{31}P or ^{1}H NMR properties of DNA[123]. In an interesting extension of the Brownian dynamics formalism which includes solvent accessible surface area, Kottalam and Case[124] computed Langevin modes of DNA hexamers. Bricki *et al.*[125] have recently reported a Brownian dynamics simulation of a B-DNA $(dA)_5.(dT)_5$ oligomer. The life time of the basepair as well as the activation energy for the opening process have been calculated which compared favourably with the corresponding experimental measurements obtained by hydrogen exchange studies.

Counterion spin relaxation of the quadrupolar nuclei in the vicinity of DNA was probed by Reddy *et al.*[37] using stochastic dynamics simulations. The calculated relaxation behaviour was found to be in qualitative accord with experiments. The results depended on the polyion conformation, fluctuations as well as small ion dynamics. The distribution and dynamics of counterions around B-DNA was reported recently by Guldbrand *et al.*[126], with a continuum solvent and an all atom model of the solute via Brownian dynamics simulations. The continuum solvent model was found to be successful in reproducing the results of the fully explicit molecular dynamics simulations for the distribution of those counterions which maintained their hydration shells. The distributions of the counterions far away from the polyelectrolyte in their simulations agreed with the results of the Poisson-Boltzmann approach.

Molecular Dynamics Simulations

These constitute a deterministic approach to the evaluation of structural, dynamic and thermodynamic properties of a given system and consist of setting up and solving numerically the Newtonian equations of motion for all degrees of freedom in the system (Eq. 7).

$$F_i = -\,(dV/dx_i) = m_i\,(d^2x_i/dt^2) \qquad \ldots(7)$$

where x_i is one of the 3N coordinates of the N particle system, m_i is the mass associated with the degree of freedom x_i, d/dt denotes time derivative, F_i is the force and V is the potential energy. This generates a trajectory in phase space which represents equilibrium fluctuations of the system in the microcanonical ensemble (*see* Refs 98, 120 and 127 for details on the theory and practice of MD simulations).

At this point in time, only a few molecular dynamics calculations have been reported on DNA systems which consider counterions and solvent water explicitly, but considering that the first MD simulation on DNA[128] was reported only ten years ago, the popularity of this technique, with simpler models for solvent and small ions, is phenomenal. Levitt's observations on the problems involved in treating electrostatic interactions in the MD simulations of DNA in vacuum are now well documented and fully supported[128]. Most studies todate treat the effect of counterions implicitly, by reducing the charges on the phosphates from –1 to –0.25, –0.32, –0.34, –0.5 (Refs 129-132) or some fractional charge justified by counterion condensation. This does serve to make the DNA electrostatically stable, but obviously lacks the correct physics. Explicit inclusion of counterions has been found to produce convergence problems in the simulations which are currently being investigated actively. Effect of the solvent and dielectric boundary are typically incorporated via a distance dependent dielectric function in a large number of studies whose justification is in the quality of the results. Furthermore, problems in analyzing molecular dynamics literature on DNA are two fold. No uniform method of analysis has been adopted by different authors to facilitate a comparison among different dynamical models. RMS deviations are too simplistic. Analysis based on Curves, Dials and Windows (CDW)[133], although too exhaustive, is helpful in a detailed characterization of the dynamical models of DNA. The other problem relates to comparison with experiment. About the DNA structure in solution, we probably do not know what is right. We do, however, seem to "know" what is wrong. Gross structural distortions, such as collapse of the groove structure, folding of the DNA may be termed incorrect but objectivity can be a casuality. Progress in the solution structure determinations via 2D-NMR combined with the information collected from crystal structures holds promise for a rigorous characterization of the different simulations on DNA. For a clear vantage point on the molecular dynamics simulations of DNA published till date, the reader is referred to the comprehensive review of Beveridge *et al.*[24].

Lee and coworkers[134] reported an MD simulation on DNA in 1984, well before the appraisal of the now most commonly used force fields[135-137]. Singh *et al.*[138] reported MD simulation studies (duration 83 ps) on d(CGCGA) based on AMBER force field[135] using a distance dependent dielectric screening function with alternative treatments of electrostatics, considering first a fully anionic form and then explicitly including "hydrated" counterions. The range of dynamic motion executed by the simulation appeared to be reasonable. The anionic model gave 9 bp/turn and the hydrated counterion model 10, the latter closer to the canonical B value[5]. The behaviour of an *in vacuo* model of the dodecamer[139] in MD simulation based on AMBER force field was recently described by Rao and Kollman[140], and Srinivasan *et al.*[141] treating the DNA together with "hydrated" counterions. It was found that over the course of *ca.* 100 ps, the dynamical structure of the system assumed an intermediate form between A and B-DNA. Trajectories initiated at either canonical B or canonical A-DNA transited to the same intermediate structural form. Fritsch and Westhof[142] investigated the three-centre hydrogen bonds in several models of dA.dT oligomers using molecular dynamics simulations with different dielectric functions in lieu of explicit solvent molecules. They reported that the calculated results were in good comparison with the experimental data based on Raman, NMR and X-ray studies when the sigmoidal dielectric function of Lavery and coworkers[83] was used in conjunction with AMBER force field.

Siebel *et al.*[143] reported an MD simulation (duration 106 ps) on the sequence d(CGCGA) with explicit consideration of 8 Na^+ counterions and 830 TIP3P water molecules in a droplet around the DNA. The simulation was said to be stable at 10 bp/turn and the average dihedral angles remained in the same range as found in the previous simulation by Singh *et al.*[138] using an implicit model for water. More tilt and twist of central base pair was found in water, and the values of glycosidic torsion angle were altered for the central C and G. The phosphate motion was, however, damped by a factor of two by the explicit water as compared with the implicit model. Sugar puckers were mixed in a statistical ratio (70-80% c2′-endo) similar to NMR results. All but two Na^+, initially at 3.1 Å, remained close to phosphate. One, however, diffused to the edge of the droplet and another appeared to be in the minor groove region. The waters were observed to form hydrogen bonds to the expected hydrophilic sites along the base pairs in major and minor grooves.

An MD simulation (80 ps) on the octamer duplex d(CGCAACGC) including 14 Na^+ and 1231 SPC water molecules under periodic boundary conditions was reported by van Gunsteren *et al.*[144] based on the GROMOS[137] force field. The simulation structure turned out to be 2.2 Å rms different than canonical B[5] and 3.5 Å rms different from the A form, i.e. intermediate between and not terribly greater than the rms of the B-form Drew and Dickerson dodecamer[139] and

canonical B. Some 80% of a set of 2D-NMR NOESY distances were satisfied by the computed average structure, considered thus to be a good candidate for solution structure of the octamer. No Na^+ ions were found to end up within 3.0 Å of any phosphate anion, leaving the sodiums and phosphates completely hydrated, and DNA solvated by water and hydrated Na^+. Swamy and Clementi[145] reported MD on B and Z-DNA in the presence of water and counterions. Calculations on G-C and A-T dodecamer sequences with 1500 water and 22 Na^+, and on a G-C dodecamer in the Z conformation with 1851 waters and 12 counterions were carried out for 4.0 and 3.5 ps, respectively, after equilibration. The run lengths were too short to permit an analysis of the structure and dynamics of DNA or the counterions/solvent molecules.

The dynamics (70 ps) of a dodecamer of poly(dG*dC) in the Z conformation including water and counterions was studied by Laaksonen *et al.*[146]. Counterions were found to preferentially coordinate with the nucleotide bases, which are pushed to the surface of the Z-form helix. Counterion diffusion was found to be lowered to a third of that in corresponding aqueous solutions of ions. Pearlman and Kollman[147] used MD simulation and free energy perturbation methods to develop an extensive theoretical account of the B to Z transition of the 5- methyl cytosine analog of (dG-dC) in the absence of added salt. In a subsequent study[148], they have examined the feasibility of generating conformational free energy maps for nucleosides. Following the work of Piccirilli *et al.*[149], Leach and Kollman[150] have examined novel nucleic acid bases and their contribution to the stability of duplex DNA, via semiempirical, molecular mechanics calculations and free energy simulations and have commented on the synergistic relationship between experiment and theory.

An MD simulation (60 ps) on the dG6*dC6 duplex with 10 sodium counterions in a droplet of 292 water molecules based on GROMOS force field was carried out by Zielinski and Shibata[151] to develop a model for the dG_n*dC_n in aqueous solutions. The hexamer maintained a B-form conformation over the course of MD, but featured high propeller twist and a surprisingly narrow minor groove. DNA-Na^+ and Na^+-Na^+ energy component terms were the major contributors to the overall shape of the potential energy profile monitored as a function of time. At about 57 ps, two sodium ions were seen to converge to an internuclear distance of 3.02 Å. The authors followed this up with another MD study[152] of the effect of GT mispairs on DNA conformation in solution. The systems simulated were $d(G_3C_3)_2$ and $d(G_3TC_2)_2$ including 10 Na^+ and first hydration shell waters.

Structure and dynamics of both water and counterions around the canonical B-form duplex of d(CGCGCGCG) were investigated recently by Forester and McDonald[10] via MD simulations. Five different simulations were carried out, one on fully charged, polyanionic DNA in pure water and four others on electrically neutral systems containing different combinations of Na^+, Ca^{2+} and Cl^- ions and water. The cations, surprisingly irrespective of their valencies, showed a strong preference for solvent separated associations with DNA and little inclination for direct site binding. The radial distribution function for Na^+-Na^+ pairs in the NaDNA system interestingly starts at about 3 Å and has maximum at 3.6 Å. This was attributed to a "water separated ion pair" arising primarily from pairs of ions that are coordinated to two different oxygens, and in the presence of added salt (NaCl) to the same oxygen on a given phosphate group.

Beveridge and coworkers have undertaken a thorough characterization of the dynamical models of DNA in aqueous solutions and reported a series of MD simulations on the duplex DNA of sequence d(CGCGAATTCGCG) including water and counterions[153-155]. The systems are typically configured in a hexagonal cell containing ~2000 waters and 22 Na^+ counterions. Results indicated that a stable B-form structure was well maintained in all the simulations. Analyses of the simulations revealed good accord with a number of detailed features seen in the X-ray crystal structure of the dodecamer, including local axis deformation near the GC/AT interfaces in the sequence and large propeller twist in the base pairs. A general conclusion emerging from these and other studies (Langley and Beveridge, private communication) is that the MD trajectories are sensitive to the initial location of counterions and overall a better agreement with crystal structure is obtained starting with a scaled phosphate charge model. A proper protocol for the treatment of counterions/electrostatics in the MD simulations on DNA and the associated structure and energetics are under active investigation in various laboratories. Miaskiewicz *et al.*[156], based on their molecular dynamics study on the Drew and Dickerson dodecamer surrounded by 22 counterions and 1431 water molecules with AMBER force field carried out for 150 ps, wonder whether longer molecular dynamics simulations would ever result in a stable unique structure of DNA. It is of course conceivable that a family of structures is compatible with the given environment and external constraints.

Experimental studies provide quite strong evidence for sequence dependent fine structure and this is a point of considerable interest for further studies on various DNA oligonucleotide sequences. As regards the role of ion atmosphere in determining the fine structure, Taylor and Hagerman[157] found recently that DNA flexibility, torsional stiffness and helical repeat were not significantly influenced by increases in the NaCl salt concentration. Light scattering experiments [158], on the other hand, showed a strong ionic strength dependence of persistence length, interpretable in terms of Manning's theory[159]. Prevost and Beveridge[160], in a bid to analyze the sensitivity of the structure of DNA to the choice of force field parameters, carried out a series of molecular dynamics computer experiments on d(CCAGGCCTGG) with CHARMm force field[135] *in vacuo*, changing, *inter alia*, charges on phosphates from -0.34 to -0.5 to -0.75, with other parameters held fixed. A CDW analysis[133] of the simulation results showed no discernible patterns. The twist and rise, in particular, seem to be uncorrelated to changes in phosphate charges. The results of both the above cited experiments and simulations based on CHARMm are quite intriguing. A clear picture on how both solvent and ionic environment influence the structural details of DNA is yet to emerge.

Applications to Protein-DNA and Drug-DNA Systems

The essential contributions to the free energy of the association of a protein or a drug molecule with DNA come from: (a) direct interactions between the protein or drug molecule and the DNA, both intra and intermolecular, (b) release of some of the water molecules bound to both protein or drug and DNA, and (c) release of some of the counterions associated with DNA. The last contribution is known as polyelectrolyte effect and is taken up for discussion in the sequel.

Polyelectrolyte Effects

Release of condensed counterions is considered to provide an entropic driving force for the DNA-ligand complex formation. A theoretical framework, for understanding the role of ion atmosphere in DNA-ligand association and to quantify the contribution of electrostatics to this process, has been developed by Manning[12] and Record *et al.*[13]. A plot of log K_{obs} versus log [MX], where K_{obs} is the observed association constant for the DNA-ligand complex and [MX] is the salt concentration, gives a slope of $-Z\psi$ (ref. 161). Here, Z is the thermodynamic equivalent of the number of counterions released during the DNA-ligand complex formation and ψ is assigned a value of 0.88 (which is a sum of 0.76, to account for the condensed counterions and 0.12, to approximate the screening effects due to the remaining uncondensed Debye-Huckel type diffuse ionic cloud in the system). The value of Z in such a log-log plot is commonly interpreted as indicating the number of ionic interactions in the complex. A small value for Z or a positive slope is taken to mean that electrostatics is not the driving force (for example, as in the binding of netropsin to DNA[162], while a large negative slope is taken to imply that electrostatics is the dominant mechanism (as in lac repressor-DNA interaction[161]. Such an analysis of the macroscopic data, however simple, elegant and useful, is not meant to give a detailed account of the molecular mechanism of DNA-ligand complexation. Theoretical methods based on molecular simulation can describe the complex in terms of intermolecular interactions, and provide a basis for developing more detailed molecular models of the process together with estimates of the corresponding energetics.

Recent free energy simulations[163] on the thermodynamics of λ repressor-operator association have shown that polyelectrolyte effects, in the region which favours protein-DNA association are short ranged. The ion atmosphere contribution to the free energy of association is favourable and is at its maximum when the protein approaches the DNA from a distance of separaton of ~7Å, which is typically the radius of the counterion condensate around B-DNA. Displacement of the condensed counterions contributes favourably to the free energy of DNA-ligand complexation. The effect known as "counterion release" in the context of DNA-ligand association, appears to be a property of the condensed counterions only. The exact magnitude of this free energy is expected to depend upon the nature of the ligand as well as the manner in which electrostatic interactions are treated and the direction of approach of the ligand towards the DNA. At this juncture, there is sufficient indication[164, 165] that theoretical calculations based on finite difference Poisson-Boltzmann methodology can quantitatively account for ion atmosphere effects on protein-DNA and drug-DNA association.

In a recent study on the binding of Tet repressor to non-specific and specific DNA monitored by stop flow technique, Kleinschmidt *et al.*[166] concluded that non-specific binding was almost completely driven by the entropy change resulting from the release of three to four Na^+ ions from the double helix upon protein binding. Formation of the specific complex was driven by a

higher entropy term resulting from the release of seven to eight Na^+ ions along with a favourable free energy term coming from nonelectrostatic interactions attributable to specific contacts. Senear and Batey[167] have since reported a thorough experimental investigation of salt effects upon the binding of λ cI repressor to the right operators (OR1, OR2 and OR3) and nonspecific DNA. The thermodynamic equivalent of the number of K^+ ions released varied as 5.9±0.7 for OR1, 6.2±0.6 for OR2, 3.8±0.7 for OR3 and 4.8±0.7 for nonspecific DNA. A transition of 0.1 *M* KCl divided condition of high salt where operator binding was highly cooperative (400-2000 fold) and of low salt where it was much less. The observed differences suggested a role for ion binding in site specific recognition and in addition to the direct contacts between the protein and DNA, indirect effects might be an important component of recognition by λ repressor. The observed differences were rationalized as reflecting different deformations of the repressor and/or different conformations of the DNA so that a different number of ions and presumably water molecules were removed from the interface upon binding.

Other Studies

Few simulation studies were conducted on protein-DNA and drug-DNA systems in aqueous solutions with small ions, largely due to heavy computational requirements. MD simulations with simpler treatment of solvent and counterions, however, have become an integral part of the X-ray and NMR structural refinement procedures[168] for the protein-DNA and drug-DNA complexes. Orozco *et al.*[169] analyzed the role of explicit solvent in modelling drug-DNA structures and advocated the usage of distance dependent dielectric constant. Swaminathan *et al.*[170] investigated the static and dynamic aspects of the intermolecular hydrogen bonding network and the organization of water via MD simulations on dCpG/proflavin complex. Gago and Richards[171] performed free energy simulations on netropsin binding to poly[d(IC)].poly[d(IC)] and poly[d(GC)].poly[d(GC)] with a consideration of explicit water and counterions. The calculated free energy change (ΔΔG) between ATAT(ICIC) sequence and GCGC sequence was 4.35 kcal/mol in close correspondence with the experimental value of 4 kcal/mol. Width of the minor groove was proposed to be a determinant of specificity in these systems. Recently, DiCapua *et al.*[172] have attempted to give a dynamical view of specificity using their MD simulations carried out with explicit solvent and counterions on λ repressor protein, operator DNA and the complex[173].

Clearly, more experimental and theoretical studies are needed and some are in progress in some laboratories (*see* for instance, Refs 174-191) to develop a comprehensive view of the molecular aspects of protein-DNA and drug-DNA recognition, DNA fine structure and the role of solvent and ion atmosphere around DNA.

Conclusion and Perspectives

Current phase of computational research may be summarized as a characterization of the force fields underlying the static and dynamical models of DNA in solution. To this extent, they are truly computer experiments to establish cause to effect relationships. These will hopefully evolve very soon into simulations with predictive value on sequence dependent fine structure of DNA, drug-DNA and protein-DNA interactions. It is obviously desirable to have molecular dynamics simulations on DNA which explicitly consider water, counterions and added salt at concentrations of physiological interest, but these are beyond the capabilities of the current generation of supercomputers. As of now, for aqueous solutions of DNA with added salt, the dielectric continuum (solvent) methods, described at length here, offer a viable alternative.

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