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The objective of this project is to carry out a critical and comparative study of the various theoretical models used for the treatment of environmental effects -- hydration and counterion atmosphere -- in molecular simulations on oligonucleotide systems. These studies form the basis for a series of collaborations with NMR spectroscopitsts and crystallographers on specific application areas of current interest in the field of nucleic acids research.

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DATE: July 1, 1988

PROGRESS REPORT ON CONTRACT: N00014-87-K-0312

PRINCIPAL INVESTIGATOR: Prof. D. L. Beveridge

CONTRACTOR: Wesleyan University

CONTRACT TITLE: Environmental Effects on Oligonucleotide Structure

STARTING DATE: June 1, 1987

Brief Summary of Project Goals

This project involves application of molecular simulation: molecular dynamics and Monte Carlo calculations to the structure, dynamics and solvation of oligonucleotide systems. Environmental effects are included in the simulations in a series of models involving progressively greater dimensionality, beginning with linear and sigmoidal forms of distance dependent dielectric functions representing the effect of water, and currently conventional screened charge models for the effect of counterions. The next level of rigor involves explicit consideration of oligonucleotide plus water with implicit (screened charge) counterion atmosphere and vice versa, explicit counterions with implicit (polarizable dielectric) water. Ultimately supercomputer simulations involving explicit representations of oligonucleotide, water and counterions will be studied, with special attention to the important but unexplored (and seemingly underappreciated) convergence issues in the calculations at this level. We hope to develop and characterize a protocol for optimum simulation methodology

required to produce reliable, reproducible results. Detailed comparison of the nucleic acid dynamics for these different environmental models will be developed also in terms of conformational and helicoidal analysis. Hydration and counterion atmosphere will be analyzed based on stereographic display of solvation density and the systematic partitioning of the complex results into well-defined contributions from the major groove, minor groove and sugar-phosphate backbone entities of the duplex. Thus a complete characterization of dynamical structure and solvation will be achieved for each system studied. These studies form the basis for a series of collaborations with NMR spectroscopists and crystallographers on specific application areas of current interest in the field of nucleic acid research.

Summary of Accomplishments: 6/1/87 - 5/31/88

In the first year of this project, two papers have been published and two more are submitted. Several additional papers are currently in preparation. In addition, two review articles on related subject areas have been prepared. In the following pages, the abstracts of the publications are collected, followed by brief descriptions of the projects currently in progress.

"Theoretical Considerations of the 'Spine of Hydration' in the Minor Groove of d(CGCGAATTCGCG)$_2$: Monte Carlo Computer Simulation," P. S. Subramanian, G. Ravishanker and D. L. Beveridge, *Proc. Natl. Acad. Sci.* **85**, 1836 (1988).

A theoretical description of aqueous hydration in the minor groove of a B-form DNA is presented here on the basis of a liquid state Monte Carlo computer simulation on a system consisting of the oligonucleotide duplex d(CGCGAATTCGCG)$_2$ in a canonical B-form together with 1777 water molecules contained in a hexagonal prism cell and treated under periodic boundary conditions. The results are analyzed in terms of solvent density distributions. The calculated minor groove solvent density shows considerable localization, indicative of discrete solvation sites and providing theoretical evidence for a well-defined ordered water structure. In the AATT sequence,

this corresponds to the 'spine of hydration' discovered by R. E. Dickerson and coworkers in the x-ray crystal structure of the dodecamer. We find however that the calculated ordered water structure also extends into the CGCG flanking sequences, supported by the N2 hydrogen bond donors and indicating that the 'spine of hydration' could thus extend throughout the minor groove of a B-form DNA. This provides a possible explanation of the positive binding entropies observed by K. J. Breslauer and coworkers for both AT and GC sequences on the complexation of netropsin to the minor groove of DNA's. Implications of these results with regard to the thermodynamic stability of DNA in water and the sequence specificity of the minor groove hydration are discussed.

"A Systematic Study of Patterns of Hydration in Nucleic Acids I. Guanine and Cytosine," H. M. Berman, A. Sowri, S. Ginell and D. L. Beveridge, *J. Biomol. Struct. Dyn.* **5**, 1101 (1988).

The hydration sites of guanine and cytosine are defined by examination of the crystal structures of bases, nucleosides, nucleotides, and three dinucleoside phosphate salts. Superposition of the results clearly reveals clusters of water molecules around certain of the heteroatoms of the bases. For the major groove region guanine, the G-N7 acceptor site shows two distinct populations of ordered water molecules, one above and one below the base plane. The G-O6 shows only one population, more or less in plane. In the minor groove region, hydration sites appear at the G-N3 acceptor site and the G-N2 hydrogen bond donor, with these positions simultaneously occupied in the crystal. For cytosine in the GpC salts, hydration was observed only at the C-O2 acceptor site in the minor groove. The observed ordered water positions for both C and G in CpG, where observed, are well localized into sites and imply markedly limited ranges for solute-solvent hydrogen bond angles. Analysis of crystals containing only single C's and G's shows a broader range of hydrogen bond angles, and no particular clustering. The patterns of hydration for two guanine and cytosine containing oligonucleotides are then predicted. The relationship between these structural motifs and thermodynamic parameters is discussed.

"Free Energy *via* Molecular Simulation: A Primer," D. L. Beveridge and F. M. DiCapua, in *Computer Simulations in Protein Engineering and Drug Design,* P. Weiner, ed., in press (1988).

The various methods for free energy determination -- e.g., thermodynamic integration, the perturbation method, and the potential-of-mean-force -- are described in an elementary treatment of the subject in the context of molecular dynamics and Monte Carlo calculations on chemical and biomolecular systems. The theory, methodology and a representative application are described in each case. The capabilities and limitations of each of the methods are delineated.

"Free Energy *via* Molecular Simulation: Applications to Biomolecular Systems," D. L. Beveridge, *Ann. Rev. Biophys. Chem.* in press (1988).

The field of free energy simulations *circa* 1988 is reviewed as applied to biomolecular systems with an emphasis on the perturbation method and the thermodynamic cycle approach to substrate and drug binding to macromolecules. Recent studies are discussed and the capabilities and limitations of the method are critically considered, with particular regard to quasi ergodic problems. Future prospects for free energy simulations vis-a-vis the supercomputer era are explored.

"Aqueous Hydration of $r(GpC)_2$ at 25°C: Monte Carlo Computer Simulation," S. Pitchumani, P. S. Subramanian, D. L. Beveridge and H. M. Berman, *Biopolymers*, submitted.

Monte Carlo calculations are described for the dinucleotide duplex $r(CpG)_2$ together with 562 TIP4P water molecules at 25°C under periodic boundary conditions. The results are analyzed based on the proximity method and computer graphic presentations of hydration density distributions. Localization of hydration density is found to correspond closely to ordered water positions in C and G containing crystal hydrates. Discrepancies of commission at C-N4 and omission at G-N3 are noted and discussed in detail.

"Convergence Characteristics of Monte Carlo Free Energy Simulations using the Perturbation Method," P. S. Subramanian and D. L. Beveridge, *Theor. Chem.*, submitted.

The convergence characteristics of Monte Carlo simulations using the perturbation method are discussed in the context of a triangular thermocycle for the $\Delta\Delta G$ of hydration for ethane, methanol and methylamine. Error bounds are critically established using the method of batch means. Independent confirmation of the $\Delta\Delta G$ for the mutation of ethane to methanol was achieved and closure of the thermocycle within 0.4 kcal/mol was obtained.

Projects in Progress, 1988-89

•Monte Carlo Calculations of Hydration Density Distributions in A, B, and Z DNA Oligonucleotides: Comparison with Crystallographically Ordered Water Sites;

•Theory and Mechanism of the Hydration-Dependent A to B Conformational Transition in DNA;

•Molecular Dynamics of the dCpG/proflavin Crystal Hydrate (collaboration with Dr. H. M. Berman, Fox Chase Cancer Center);

•Monte Carlo Simulation of Counterion Condensation on DNA: Dielectric Function, Cation Type and Salt Dependence;

•An Atomic Solvation Theory for Nucleic Acids;

•Graphical Analysis of Microscopic Details of DNA Dynamics in Conformational and Helicoidal Coordinate Space (collaboration with Dr. R. Lavery, Institut de Biologie Physico-Chimique, Paris);

•Characterization of the Molecular Dynamics of Oligonucleotide Double Helices as Described by AMBER, CHARMm and GROMOS Force Fields; Influence of Dielectric, Counterion and Hydration Models;

•Determination of Oligonucleotide Structure in Solution Using 2D-NOE Distance Information from NMR and Restrained Molecular Dynamics Calculations (collaboration with Prof. P. H. Bolton);

•Effects of Hydration, Salt and Flanking Sequence Variations on the Structure and Dynamics of the Eco R1 Binding Site in the d(CGCGAATTCGCG) Duplex: Comparison of Results with NMR Studies (collaboration with Prof. I. Russu);

•Theoretical Studies of Molecular Recognition and Subunit Assembly Processes in ATCase (collaboration with Prof. N. Allewell).