SIMULATION OF BIOMOLECULAR DIFFUSION AND COMPLEX FORMATION

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ABSTRACT Diffusion is a phenomenon of very widespread importance in molecular biophysics. Diffusion can determine the rates and character of the assembly of multisubunit structures, the binding of ligands to receptors, and the internal motions of molecules and assemblies that involve solvent surface displacements. Current computer simulation techniques provide much more detailed descriptions of diffusional processes than have been available in the past. Models can be constructed to include such realistic features as structural subunits at the submolecular level (domains, monomers, or atoms); detailed electrostatic charge distributions and corresponding solvent-screened inter- and intramolecular interactions; and hydrodynamic interactions. The trajectories can be analyzed either to provide direct information on biomolecular function (e.g., the bimolecular rate constant for formation of an electron-transfer complex between two proteins), or to provide or test models for the interpretation of experimental data (e.g., the time dependence of fluorescence depolarization for segments of DNA). Here, we first review the theory of diffusional simulations, with special emphasis on new techniques such as those for obtaining transport properties of flexible assemblies and rate constants of diffusion-controlled reactions. Then we survey a variety of recent applications, including studies of large-scale motion in DNA segments and substrate "steering" in enzyme-substrate binding. We conclude with a discussion of current work (e.g., formation of protein complexes) and possible areas for future work.

INTRODUCTION

The theoretical study of the motion of biological molecules is emerging as an important field of molecular biology. Two complementary techniques used to study these motions are molecular dynamics and Brownian dynamics. In molecular dynamics, a computer is used to solve the Newtonian equations of motion for the atoms in a system of interest for a finite period of time. The method has been applied to proteins, nucleic acids, and other biological molecules. These calculations have provided many fundamental new insights into the nature of biological molecules, as discussed in a number of reviews (1, 2).

One of the limitations of the standard molecular dynamics method is that only short time periods, usually less than a nanosecond, are accessible on present-day computers. The time ranges explored by relaxation techniques such as nuclear magnetic resonance (NMR), dynamic light scattering, and electric birefringence are much longer. Furthermore, most biological activity occurs over longer time periods. The rates of many biochemical processes depend on the frequency with which reaction partners encounter each other in solution (3–8). Examples of such "diffusion controlled" processes are known in the areas of enzyme-substrate catalysis, antibody-antigen binding, protein-DNA interaction, etc. (7). Diffusion is an intrinsically slow process. The time required for even a small molecule to diffuse 5 Å in water may exceed 500 ps. The technique of Brownian dynamics, which is based on diffusion or Langevin equations, can be used to simulate the long-time dynamical behavior of model systems (9).

In Brownian dynamics, a simplified model is used to represent the actual system, although the investigator has considerable freedom in its design. A series of increasingly realistic models of a particular system can be studied in a systematic way. A protein, for example, can be modeled as a single sphere derived from its hydrodynamic radius (10, 11), or as an array of spheres, each of which might correspond to a rigid domain or residue of the protein (12). Similarly, a DNA restriction fragment can be modeled as a stiff string of touching beads (13). One can include in a straightforward manner forces arising from electrostatic interactions between charged subunits; stretching, bending, and constraint forces in semirigid arrays of subunits; and other interactions. The solvent is represented as a viscous continuum that exerts stochastic forces on the model subunits. Solvent structural features (e.g., screening of Coulombic interactions by mobile ions) can be incorporated through appropriate potentials of mean force for the subunit interactions.

The Brownian dynamics method has two limitations relative to molecular dynamics. First, because solvent-averaged potentials are used, one cannot obtain detailed information on solvation structures (e.g., the pattern of

hydrogen bonds in water molecules around a solute). The average effects of such structures can, however, be incorporated into the potentials of mean force. Second, one cannot get information on the details of inertial motions that are evident during very short intervals of time, because the underlying diffusion equations describe the average motion of solutes whose motions have been interrupted by at least a few collisions with solvent molecules. Brownian dynamics gives a reliable description of solute motions for times longer than the solute momentum relaxation time (9); for typical biopolymer systems, this is <0.1 ps (12).

The most direct application of Brownian dynamics involves the determination of transport coefficients (diffusion constants) for rigid and semiflexible structures. Several numerical methods are available for determining transport coefficients of rigid structures modeled as arrays of spheres (14), but this is not the case for flexible structures. However, analytical techniques developed by a number of investigators are appropriate for certain classes of problems (15, 16). Transport properties for models of arbitrary complexity can be obtained from Brownian dynamics simulation. These are obtained either by carrying out a large number of single dynamics step "trajectories" starting from representative initial configurations followed by averaging the appropriate displacements (17), or by averaging over trajectories that are propagated for longer periods of time (13, 17–18).

In this discussion, we consider two examples of the application of Brownian dynamics to study internal or relative motions of biological molecules. In each case, the model system is allowed to evolve with time by taking successive dynamics steps to generate a trajectory (13). In the first example, relaxation "experiments" (fluorescence depolarization and depolarized light scattering) are simulated by appropriate averaging of a large number of trajectories. The system studied is a DNA restriction fragment modeled as a stiff string of touching beads. In the second example, the same basic procedure is used, but applied to bimolecular diffusion-controlled reactions. From a large number of Brownian dynamics trajectories, one obtains a recombination probability for two reactive species that start at some initial separation. This recombination probability can then be related directly to a rate constant (19). This method is applied to the diffusion-controlled enzyme-substrate reaction between superoxide dismutase and superoxide.

THEORY

Brownian dynamics is a method that allows one to simulate the diffusional motion of an assembly of interacting solute molecules. Consider first the simple case of an isolated spherical molecule in the absence of any direct force. If the particle were initially located at some point $\mathbf{R}^0$, then the probability density, $\rho(\mathbf{R}, \Delta t)$, of finding it at $\mathbf{R}$ after time Δt is given by

$$\rho(\mathbf{R}, \Delta t) = (4\pi D\Delta t)^{-3/2} \exp\left(-(\mathbf{R} - \mathbf{R}^0)^2/4D\Delta t\right), \quad (1)$$

where D is the translational diffusion constant of the molecule. In a simulation, the new position of the particle is selected at random from this Gaussian distribution. If a large number of steps using the same $\mathbf{R}^0$ and Δt for each were carried out, then the distribution of final positions must obey Eq. (1) above. The first step of the diffusional trajectory is then

$$\mathbf{R} = \mathbf{R}^0 + \mathbf{S}, \quad (2)$$

where $\mathbf{S}$ is a vector of Gaussian random numbers. The components of $\mathbf{S}$ have zero mean ($\langle S_\alpha \rangle = 0$; $\alpha = x$, y, or z) and have the variance

$$\langle S_\alpha S_\beta \rangle = 2D\delta_{\alpha\beta}\Delta t, \quad (3)$$

where $\delta_{\alpha\beta}$ is the Kronecker delta. $\delta_{\alpha\beta} = 1$ if $\alpha = \beta$ and equals 0 otherwise. Physically, $\mathbf{S}$ represents the stochastic displacement of the spherical molecule resulting from collisions with solvent. A trajectory can be extended to longer times ($2\Delta t$, $3\Delta t$, etc.) by repeated application of this algorithm with each step beginning at the position chosen in the previous step. By computing a large number of such trajectories with different random numbers, one generates a description of how an ensemble of diffusing molecules behaves.

When direct forces act on an isolated spherical particle (such as the centrifugal force on a sedimenting globular protein), it is necessary to account for the displacement that arises as a result of these forces. Eq. 2 is replaced with

$$\mathbf{R} = \mathbf{R}^0 + \mathbf{S} + \mathbf{F}^0\Delta t/f = \mathbf{R}^0 + \mathbf{S} + \frac{\Delta t}{k_B T} D\,\mathbf{F}^0, \quad (4)$$

where f is the friction constant, k_B is Boltzmann's constant, T is the absolute temperature, and $\mathbf{F}^0$ is the initial direct force on the molecule. A single dynamics step should be short enough so that $\mathbf{F}$ remains essentially constant as the molecule is displaced from $\mathbf{R}^0$ to $\mathbf{R}$ in time Δt.

When more than one spherical molecule or particle is present, they interact indirectly with each other by perturbing the velocity of the intervening solvent (hydrodynamic interaction) and perhaps directly through direct forces. A number of Brownian dynamics algorithms are available (9, 20–21), but in this work the algorithm of Ermak and McCammon is used (9). For a system of N interacting spherical subunits, the position of subunit i, $\mathbf{R}_i$, after a dynamics step of duration Δt, is given by

$$\mathbf{R}_i = \mathbf{R}_i^0 + \mathbf{S}_i + \frac{\Delta t}{k_B T}\sum_{j=1}^{N} \mathbf{D}_{ij}^0 \cdot \mathbf{F}_j^0, \quad (5)$$

where $\mathbf{R}_i^0$ is the initial position of subunit i. As in the single particle case, displacement results from direct forces ($\mathbf{F}$) and solvent collisions ($\mathbf{S}$). However, the diffusion constant of Eq. (4) is replaced by a generalized diffusion tensor, $\boldsymbol{D}$. These tensors represent the coupling of the motions of different subunits by hydrodynamic interaction (HI). As in the case of a single particle, the mean of the stochastic displacements is zero, but the generalized variance must satisfy the following condition

$$\langle \mathbf{S}_i \mathbf{S}_j \rangle = 2\,\boldsymbol{D}_{ij}\Delta t. \quad (6)$$

Methods of constructing $\mathbf{S}_i$ are described elsewhere (9, 20).

As a lowest-order approximation, HI between different subunits can be ignored completely. In this case $\boldsymbol{D}_{ij} = D_i\,\delta_{ij}\,\boldsymbol{I}$ where $\boldsymbol{I}$ is a 3×3 identity tensor and D_i is the translational diffusion constant of subunit i. For a relatively large, neutral sphere, the Stokes-Einstein equation gives

$$D_i = k_B T/6\,\pi\,\eta\,a_i, \quad (7)$$

where a_i is the radius of subunit i and η is the solvent viscosity. For small molecules (comparable to the size of the solvent molecules) (22) or molecules that interact strongly with the solvent (ions in water, for

example) (23), the observed diffusion constant may differ from the Stokes-Einstein value by a numerical factor that is usually in the range 0.5 to 2.0.

At the higher level, HI can be approximated using the Oseen (24) or Rotne-Prager (25) tensors. For identical nonoverlapping spheres of radius a, the latter tensor is given by

$$\mathbf{D}_{ij} = \frac{k_B T}{6\pi\eta a}\mathbf{I} = \mathrm{D}_0\,\mathbf{I} \qquad (i = j)$$

$$\mathbf{D}_{ij} = \frac{3aD_0}{4\mathrm{R}}\left[\left(\mathbf{I} + \frac{\mathbf{R}\,\mathbf{R}}{R^2}\right) + \frac{2a^2}{R^2}\left(\frac{1}{3}\mathbf{I} - \frac{\mathbf{R}\,\mathbf{R}}{R^2}\right)\right](\mathrm{i} \neq \mathrm{j}) \qquad (8)$$

where $R = |\mathbf{R}_i - \mathbf{R}_j|$. The Oseen tensor (stick boundary conditions) is obtained by omitting the a^2/R^2 terms on the right-hand side of Eq. 8. Other tensors (Oseen [26], Rotne-Prager for overlapping spheres [14, 25], as well as higher order [27]) are described elsewhere. For different subunits, it can be seen from Eq. 8 that $\boldsymbol{D}_{ij}$ falls off as a/R. If the subunits are far apart, HI is small.

Attention shall now be turned to the problem of obtaining a bimolecular rate constant for diffusion-controlled reactions by Brownian dynamics simulation. Smoluchowski and Debye investigated the problem of diffusion-controlled reactions between uniformly reactive spheres in the absence and presence of centrosymmetric Coulombic forces (4). More recently, there has been a proliferation of theoretical studies based on more refined models, as described in recent reviews (6–8). Perhaps the most advanced analytical-numerical methods are those based on the formalisms of Wilemski and Fixman (28), Keizer (29), and Zeintra et al. (30). The Brownian dynamics simulation method is sufficiently general to model systems of arbitrary configurational complexity and arbitrary inter- and intramolecular forces; and it allows for inclusion of hydrodynamic interaction. When a variety of interactions are present between the reactive species, there is probably little hope of obtaining analytical rate constants at a detailed level and recourse to simulation methods becomes necessary.

As an example, one can imagine generating diffusional trajectories of a substrate relative to an enzyme target. From the frequency of collisions of properly oriented substrates with the active site of the enzyme, a rate constant could then be calculated. In practice, this approach encounters the difficulty that many trajectories wander far from the enzyme. To determine the ultimate fate of such trajectories (whether they return and lead to reaction, or escape reaction altogether) would require infinitely long simulations. Recently, Northrup et al. have devised a method to circumvent this difficulty (19). The diffusion space around the enzyme is divided into two regions. The "inner" region is finite and comprises that volume adjacent to the enzyme in which the interactions are complicated and best dealt with numerically. The "outer" region is of infinite volume but is everywhere far enough from the enzyme so that the diffusional behavior can be described analytically. Trajectories then need be computed only in the finite inner regions. Let the target (enzyme) be surrounded by a spherical surface of radius b which lies just outside the inner region. Then the rate constant, k, can be written

$$k = k_D(b)\,p, \qquad (9)$$

where p is the probability that the reactant pair, starting at initial separation $r = b$, will ultimately react, and $k_D(b)$ is the steady-state rate at which reactants with separation $r > b$ first strike the b-surface. Because of the restrictions placed on b, $k_D(b)$ can be determined analytically (6)

$$k_D(b) = \left(\int_b^\infty dr\left[\frac{\exp[u(r)/k_B T]}{4\pi r^2 D(r)}\right]\right)^{-1}, \qquad (10)$$

where $u(r)$ is the (centrosymmetric) potential of mean force (i.e., the effective interaction energy between enzyme and substrate), and $D(r)$ is the relative diffusion constant. In the special case of no hydrodynamic interaction

$$D(r) = D_{rel} = D_1 + D_2, \qquad (11)$$

where D_1 and D_2 are the translational diffusion constants of the individual reactants.

To avoid the problem of reactants initially at $r = b$ diffusing to large distances, trajectories are terminated if r exceeds some cutoff distance $q > b$. What is actually determined in a simulation over many trajectories is a recombination probability, β, which is different from the desired p. This is because it is conceivable that a trajectory with $r > q$ would eventually react if it were not terminated at $r = q$. Fortunately, it is possible to correct β to obtain p using branching arguments; it can be shown that

$$p = \frac{\beta}{1 - (1 - \beta)k_D(b)/k_D(q)}. \qquad (12)$$

This point is discussed in more detail elsewhere (19).

RESULTS

Relaxation Experiments on DNA Restriction Fragments

The DNA molecule is modeled as a string of N touching beads of radius a linked end-to-end by $N - 1$ virtual bonds. Following Hagerman and Zimm (31), a bead radius of 15.9 Å was used because this yields a structure that mimics the overall hydrodynamic behavior of a continuous worm-like chain cylinder with radius 13 Å (corresponding to DNA). Bending forces were derived from the potential (32)

$$U_{bend} = \frac{g}{2}\sum_{j=1}^{N-2}\theta_j^2, \qquad (13)$$

where g is the bending force constant and θ_j is the angle between virtual bond vectors j and $j + 1$. The persistence length, P, is related to g by the expression (32)

$$g = Pk_B T/2a. \qquad (14)$$

In this work, P is varied from 200 to 800 Å and $N = 30$, corresponding to a fragment 922 Å long. Stretching forces that hold neighboring beads at a nearly constant separation of $2a$ are introduced using a displaced quadratic potential with a stiff force constant. This was found to yield results identical with earlier studies where fixed bond length constraints were used (13), but the present method is computationally more efficient. HI was included in the simulation using the Rotne-Prager tensor (Eq. 8), but these tensors were "preaveraged" to avoid the necessity of recomputing $\boldsymbol{D}_{ij}$ repetitively as the molecules deform during dynamics. In a comparative study of 10 subunit chains, it was found that "experiments" on preaveraged and nonpreaveraged chains were essentially identical.

To simulate fluorescence depolarization (fd), it is assumed that a dye molecule is rigidly attached to the structure. For the sake of illustration, the dye is placed near the center of the chain with its emission dipole colinear with a virtual bond vector. The polarization

anisotropy is then given by (13, 33)

$$r(t) = 0.4 \langle P_2[\mathbf{u}(t) \cdot \mathbf{u}(0)] \rangle$$
$$= 0.4 \exp [-3 \langle \theta^2(t) \rangle / 2], \quad (15)$$

where P_2 is a Legendre polynomial, $\mathbf{u}(t)$ is a unit vector along a particular bond of the chain at time t, and $\langle \theta^2(t) \rangle$ denotes the mean square angular displacement of that unit vector. This is the average that must be determined to simulate fd. For a rigid structure, $\langle \theta^2(t) \rangle = 4 \langle D_R \rangle t$ where brackets denote the average over a large number of structures and D_R is the rotational diffusion constant about an axis perpendicular to $\mathbf{u}$. For a flexible structure, $\langle \theta^2(t) \rangle$ is a complicated function of time. In depolarized light scattering, the relevant average is a reduced dynamic structure factor defined by (34)

$$g(t) = (N-1)^{-2} \sum_{i,j-1}^{N-1} \langle P_2[\mathbf{u}_i(t) \cdot \mathbf{u}_j(0)] \rangle, \quad (16)$$

where $\mathbf{u}_i(t)$ is the unit vector along the ith bond at time t. This is similar to the expression for $r(t)$, except that cross correlations between all virtual bonds, corresponding to different anisotropic scattering elements, are included in the average. In the special case of rigid structures, this reduces to

$$g(t) = g(0) \exp [-6 \langle D_r \rangle t], \quad (17)$$

which is identical to $r(t)$ except for a constant scaling factor. This is not the case, however, for flexible DNA fragments, as shown in Figs. 1 and 2 (note the different vertical scales). These two "experiments" were carried out using the same simulation of 280 trajectories selected at random from a Boltzmann distribution of starting configurations. Evidently, fd is more sensitive to rapid internal motions even though both experiments reveal flexibility on the time scale 0 to 200 ns. If the DNA fragments were behaving as rigid bodies, the dotted lines on Figs. 1 and 2 would have been observed corresponding to $\langle D_R \rangle = 2.5 \times 10^4$ s (31). The strong dependence of fd on flexibility is shown in Fig. 3. The stiffer the molecule, the lower the depolarization at both short and long times. The long-time behavior can be readily understood, because a stiff molecule has a larger average end-to-end distance and hence a smaller rotational diffusion constant (31). These results and others will be described more fully in a future publication.[1]

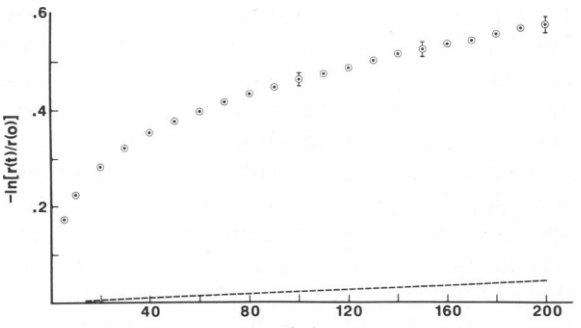

FIGURE 1 Fluorescence depolarization of a short wormlike chain: P = 400 Å, L = 922 Å. The transition moment lies along the local symmetry axis of the chain located near its center. The dotted line represents the behavior expected of an ensemble of rigid chains with the same P and L.

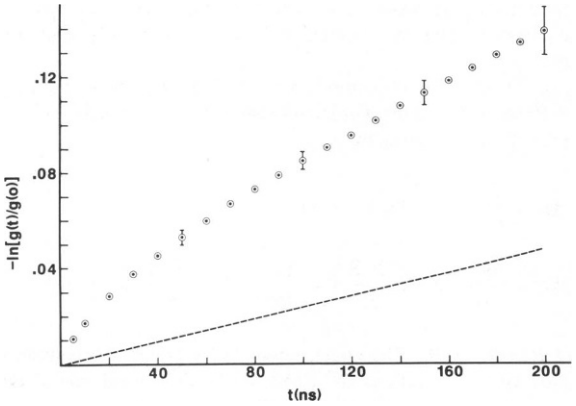

FIGURE 2 Depolarized light scattering (Eq. 16) of a short wormlike chain: P = 400 Å, L = 922 Å. Details in Fig. 1.

The error bars on selected data points were obtained from standard deviations of equivalent but independent subsimulations. For example, the 280 trajectories of Figs. 1 and 2 represent seven subsimulations of 40 trajectories each. These results required ~25 h of CPU time on a UNIVAC 1100 computer. However, we anticipate this could be reduced by a factor of 100 using a CYBER 205 supercomputer.

Diffusion-Controlled Reaction Between Superoxide and Superoxide Dismutase

Electrostatic interactions influence the rates of many biomolecular associations (4). For example, the charge distribution of a particular enzyme-substrate system may help to draw the two species together and "steer" them into a proper relative orientation for a catalytic reaction. Particularly interesting in this regard is the diffusion-controlled transformation of superoxide (O_2^-) catalyzed by the enzyme copper, zinc superoxide dismutase (SOD) (35, 36). The rate constant for this transformation has the unusual feature of decreasing with increasing salt concentration despite the fact that both species are negatively charged at neutral pH (37). It has been argued that these results are due to the noncentrosymmetric charge distribution of the dimeric enzyme (36).

In initial studies (10, 11), the SOD dimer and O_2^- were modeled as spheres with radii 28.5 and 1.5 Å. Two reactive

[1]Allison, S. A., "Brownian Dynamics Stimulation of Wormlike Chains, Fluorescence Depolarization and Depolarized Light Scattering," submitted to Macromolecules.

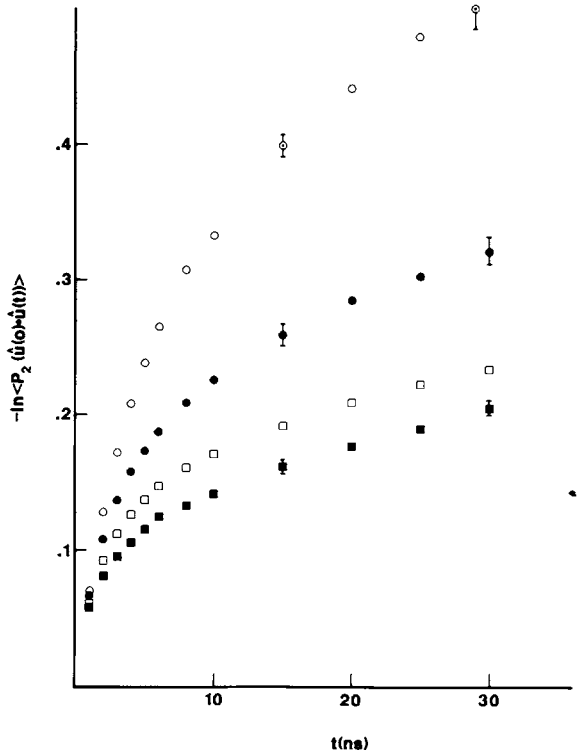

FIGURE 3 Effect of P on fluorescence depolarization from 30 subunit wormlike chains. **u** is a unit vector located at the chain center. P (Å) = 200 (○), 400 (●), 600 (□), 800 (■).

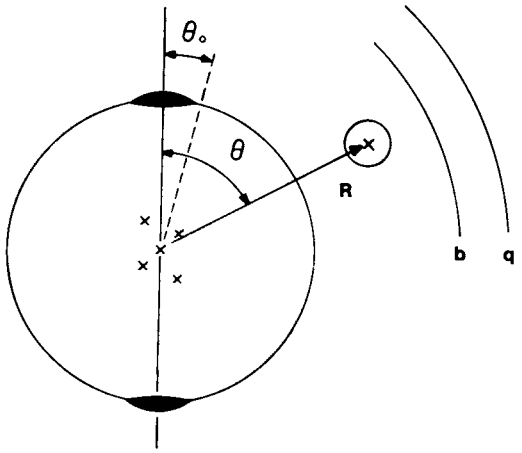

FIGURE 4 Schematic illustration of the superoxide dismutase O_2^- model. Crosses indicate positions of several charges. Active sites are indicated by the dark caps on the SOD sphere; $\theta_0 = 10°$.

patches corresponding to the active site regions of SOD were defined by surface points within 10° of an axis running through the center of the sphere (Fig. 4). Trajectories were usually initiated at b = 300 Å and terminated after collision with the active site or with a truncation sphere at q = 500 Å. A series of increasingly realistic electrostatic models was studied as summarized below.

(*A*) *One-Charge Model*: A single charge of −4 was placed at the center of the enzyme to represent the net charge.

(*B*) *Three-Charge Model*: Derived from Model C below by angular averaging the electrostatic potential about the axis passing through the center of the two reactive patches.

(*C*) *Five-Charge Model*: Designed to reproduce the monopole, dipole, and quadrupole moments associated with the charged groups in the x-ray structure of SOD (38).

(*D*) *76-Charge Model*: Charges were placed at the crystallographic coordinates of the 76-charged residues of SOD dimer.

(*E*) *2196-Charge Model*: Partial charges were assigned to all nonhydrogen atoms of the SOD dimer.

A dielectric constant of 78 was assumed throughout. Also, HI was ignored since it was previously found to have little effect on "steering" even though it does reduce the rate (39). Reduced rate constants are given in Table I ($X = k/k^0$, where $k^0 = 4\pi D_{rel}$ [30 Å]) for the different electrostatic models in the absence of added salt. Note that the rate for the single charge model is significantly lower than the other rates, which shows that the charge distribution of the enyzme does indeed steer superoxide toward the active site. Surprisingly, models *B* through *E* yield essentially the same rate. Although the charge distribution leads to a rate enhancement, it is the long range character of this distribution that affects the rate in the case of SOD.

Salt effects can be represented using simple Debye-Hückel type models for point or finite ions. Using model *C*, the reaction rate first increased, and then decreased to a plateau as the solvent ionic strength was increased (11). The initial behavior at low salt can be attributed to screening out repulsive net charge (monopole) interactions. At higher salt, where the shorter-ranged attractive forces are screened, this trend is reversed.

The initial studies are currently being extended in a number of ways. These include improvements in the dielectric model, more realistic treatment of solvent ions, and accounting more accurately for the surface topography of the enzyme. Generalizations of the original method (19) have also made it possible to initiate trajectories with the two reactive species in closer proximity (40).

TABLE I
REDUCED RATES FOR VARIOUS ELECTROSTATIC MODELS OF ZINC SUPEROXIDE DISMUTASE (SOD)

Model	No. of charges	X
A	1	0.056 ± 0.004
B	3	0.074 ± 0.004
C	5	0.079 ± 0.005
D	76	0.082 ± 0.011
E	2196	0.080 ± 0.006

CONCLUSION

Simulation methods are expected to open the way for detailed study of a wide variety of diffusional phenomena in cellular and molecular biology. The internal motions of flexible structures such as immunoglobulins or myosin as well as fluorescence energy transfer between donors and acceptors on the same or different molecules (as in a flexible polymer) could be studied by the methods described here. Studies of enzyme-substrate binding can be extended to predict the effects of amino acid sequence changes. Other refinements might include incorporation of internal flexibility of enzyme or substrate that would modulate the reactivity of active sites. The association of protein or protein-DNA complexes can be studied as a straightforward extension of the work on SOD. Other simple association phenomena (e.g., antigen-antibody, hormone-receptor) can be handled in the same way. The increasing availability of supercomputers will make substantially more sophisticated modeling possible in the future.

S. A. Allison is the recipient of a Presidential Young Investigator Award, a Camille and Henry Dreyfus Grant for Newly Appointed Faculty in Chemistry, and a grant from the Research Corporation. S. H. Northrup is the recipient of a National Institutes of Health (NIH) Research Career Development Award (AM01403) and a grant from the Petroleum Research Fund. J. A. McCammon is an Alfred P. Sloan Research Fellow and is the recipient of an NIH Research Career Development Award (HL00664) and a Camille and Henry Dreyfus Teacher-Scholar Award. The research described here was supported in part by grants to the University of Houston from NIH (GM31749); the National Science Foundation (PCM8201204, PCM8404219); and the Robert A. Welch Foundation.

Received for publication 22 April 1985 and in revised form 31 May 1985.

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DISCUSSION

Session chairman: Adrian Parsegian
Scribes: Gary A. Griess and Eric T. Baldwin

BLOOMFIELD: The general technique of Brownian dynamic simulation is an attractive procedure that gets into the range of times where we do most of our experiments. What does diffusional simulation leave out (that molecular dynamics would include if it could be carried out), that would be important for analysis of experiments on the nanosecond and longer time scale? Also, can you estimate a lower bound to the time scale of Brownian dynamics? Does leaving out the velocity make a difference?

ALLISON: I think you can get to all times by overlapping molecular dynamics and Brownian dynamics. For example, molecular dynamics can get out to a couple hundred picoseconds. Brownian dynamics is applicable on time scales longer than the momentum relaxation times of the solvent, and this is on the order of a few tenths of a picosecond. Hence the two methods overlap. As for what is left out, Brownian dynamics replaces the solvent with a bath of random noise. The potentials are not real potentials but potentials of mean force. So you lose the detailed atomic description of the solvent when you go to Brownian dynamics.

BLOOMFIELD: What is your sense of the consequences of that particular omission for the valid analysis of physical situations?

ALLISON: When you ignore momentum relaxation but correct for it using random numbers to represent stochastic displacements, you must remember that the dynamics are being generated in a statistical rather than deterministic sense.

BLOOMFIELD: Macromolecular interactions depend strongly on water structure and its adjustment to the polymer's approach. Do you see any way of incorporating solvent into Brownian dynamics? What might its neglect leave out? The effective dielectric constant for electrostatic interactions is a related problem.

ALLISON: In Brownian dynamics, simulation of the diffusion-controlled reaction between the enzyme and the substrate the dielectric constant was set at 78, and this would certainly not be true if you were looking at the effective dielectric constant between two groups inside a protein. However, in this case, over much of the diffusional process the enzyme and substrate are separated by a fairly thick layer of water. To assume a bulk dielectric constant of water would be fairly accurate when enzyme and substrate are far apart. Presently, the Warwicker-Watson model, where you model the protein as one dielectric and the water as another dielectric, is being used to develop a more realistic model for this problem. This work is just getting under way, and we have no results yet. Our philosophy is to start with the simplest model and develop more and more sophisticated models. If the simple model works, that model should be used.

EISENBERG: In regard to the flexible DNA worm-like chain, I would like to know whether you can interpret some experiments which are well established (Kam, Borochov, and Eisenberg. 1981. *Biopolymers*. 20:2671–2690). The apparent diffusion constant, D_{app}, from quasielastic light scattering, yields the translational diffusion constant at low values of the scattering vector **q**, but increases in sigmoidal fashion with increasing values of **q**. If you stiffen up the molecule, can you see changes in the predicted relaxation times?

ALLISON: Yes, you can carry out the simulations over both high and low scattering vectors. Different experiments correspond to different averages over the internal coordinates of the worm-like chain. You would have to carry out the average over the appropriate physical quantity. I have done that for the 30 subunit worm-like chains, but the results are not particularly interesting. Polarized light scattering is not very sensitive to internal motions of 30 subunit worm-like chains unless the scattering vector is very large. You get a diffusion constant corresponding to that of the overall molecule. Different relaxation times depend on chain conformation and not on internal bending.

POTSCHKA: In macromolecules the location of the target of a reaction-diffusion process is usually quite different from the center of the molecule. Compared to the properties of the target the remainder of the molecule most often has only second-order influence via rotational diffusion. You interpret differences between a simple charge vs. five charge centers by the importance of multipole moments. Intuitively this should be a matter of radial distance away from the reaction center. Wouldn't a simpler model centered in the target do equally well?

ALLISON: The model of SOD enzyme has two active patches. If you put a charge at the center you have a charge monopole model. The five-charge model has charges pulled back inside the enzyme. Now, if you keep the quadrupole moment constant and move quadrupolar charges farther out ($\mathbf{q}a^2$ = constant, where **q** is the quadrupole charge and a the charge separation) the electrostatic potential doesn't change appreciably, as long as the quadrupolar charges are kept within the protein interior.

LEE: I would like to elaborate on Victor Bloomfield's question. Adrian Parsegian, Donald Rau, and I have measured a hydration force that

depends on perturbation of water out to ~10 Å from the surface of DNA double helices. When you have electrostatic forces, the perturbation giving the hydration force must be included in any electrostatic picture, effectively changing the dielectric constant of water.

ALLISON: They may well be important. How can you improve the model to account in a better way for the dielectric constant for the water?

PARSEGIAN: The preoccupation with the dielectric constant as the right fudge factor may be erroneous. There may be another kind of physics of interaction, the physics of dehydration. So it is not just a matter of manipulating the ϵ or D when you want to study the interactions between bodies. Don't stick to old parameters; there might be new ones to worry about.

TAINER: I have two related points regarding superoxide dismutase (SOD). First, if you consider the enzyme as a sphere it seems to me you are already taking for granted local steering effects because the active site is actually located ~14 Å beneath the surface of your sphere. So you are assuming that any time the superoxide reaches the sphere above the active site you have a productive collision. Second, I am bothered that your five-charge model works so well, and I wonder what that is telling us. There is no evidence of different rates among the SOD enzymes from different sources. By your arguments, it seems that the rate should vary considerably as the charge changes. Local charges must be important, because the the overall charge changes, and, as far as I know, the evidence is that the rate is constant.

ALLISON: The objective was to see if the charge distribution does steer superoxide into the active site. We are not claiming that the particular numbers we are getting are quantitatively accurate, but we think that on the basis of the model we are using, we can say that the charge distribution is guiding the superoxide into the active site of the molecule. The rate constants are three to four times larger than experimental rates, so our models are still too simple. There are a variety of ways to improve that. We are assuming that once superoxide gets inside a patch it reacts. That is probably not true because undoubtedly some SO gets in and then escapes.

GLUCKSMAN: Going back to DNA, how can you extrapolate from a naked piece of DNA, 30 bases long, to a piece of chromatin coated with histones?

ALLISON: These chains are ~920 Å long. Each subunit is ~30 Å in diameter. This is the model of Hagerman and Zimm (reference 31) who found that a bead model could reproduce the overall hydrodynamics of a continuous cylinder model of a wormlike chain, provided two criteria were met: first, that the lengths were the same, and second, that the volume of the continous cylinder and discrete bead models were the same. That is where the 31.8 Å comes up for size of these subunits. Each of these is not a single base pair but a hydrodynamic element.

BLOOMFIELD: The apparent hydrated diameter of DNA from sedimentation measurements is ~27 Å. The bare P–P distance is 20 Å. You are a little broader than that, but it is in the same ballpark. I wanted to get back to Heini Eisenberg's discussion because a misconception may have been propagated with regard to time scales. When you are in that low angle-low $\mathbf{q}^2$ plateau region, it is by no means in the nanosecond time range, but in milliseconds for translational diffusion. When you go up the plateau to higher $\mathbf{q}^2$, as Eisenberg says, the mechanism of that transition is not terribly well understood. One explanation is that you're looking at some internal modes. Mickey Schurr has suggested that you are looking at segmental diffusion within the DNA coil. Those time scales are probably microseconds. My question is, to what extent are your capabilities up to that type motion of segmental flex? This again would be polarized scattering, not depolarized scattering.

ALLISON: At Georgia State the computer capabilities on a mainframe UNIVAX 1100 are ~1%, the capacity of a supercomputer (cyber 205). We get out to ~200 nanoseconds. We can't get out to microseconds. This is in a Brownian dynamic simulation for 30 subunit wormlike chains with pre-averaged hydrodynamic interactions. On a supercomputer you could do microsecond simulations of polarized light scattering for 30 subunit wormlike chains, but much longer chains would be difficult. One way to extend this would be to use something like a Harris-Hearst model instead of a discrete wormlike chain of touching beads in which larger subunits are used. In this model you have bending forces and stretching forces. Now you are going to lose some of the rapid internal motion when you go to those lower resolution models, but I have a feeling that for polarized light scattering you are not going to lose much, because those experiments are going to be insensitive to extremely rapid motion.

SCHOENBORN: Dielectric constants are macroscopic constants which you are using on an atomic scale (microcanonical ensemble). What does a dielectric constant mean on a 2 Å scale in a protein or DNA?

ALLISON: Because temperature and not total energy are constant the ensemble is canonical.

BLUM: In regard to SOD and the charge distribution, and the dielectric constant, I have a feeling that when you looked at the multipole solutions you made a very large simplification in your picture of charge distribution. The incoming superoxide anions see on the globular surface of SOD the whole pattern of clusters of charges superimposed on the pattern of hydrophilic and hydrophobic patches on the surface. Only by mapping the surface charge distribution can you really approach the situation. To look at multipole charges you must look on a gradient of dielectric constants between four and 78 in the outer shell of protein globule.

The other interesting thing in your approach is the choice of SO anion, because it is a small charged particle that can see details on the protein surface. From the point of view of the solvent, you are treating SO anion with the solvent value of the dielectric constant, but when it comes close to the surface of the protein you cannot ignore the change of dielectric constant. Do you agree?

ALLISON: For the process we are looking at, the rate constant for association, I believe it is the long-range interactions that are dominating that process.

BLUM: As long as you look at one charge, you are right. When you look at the multipole, it is different.

ALLISON: You are saying that the multipole polarizes the surface charge on the enzyme. We are starting to modify the calculations to use different dielectric constants inside and outside the sphere. Perhaps some of these effects will be incorporated in future simulations.

SALEMME: We have found that the major factor in the rate enhancement is the resolution of the solid angle that has to be sampled to form a

reactive complex. The actual reaction rate between two colliding molecules with localized reactive surfaces depends on the probability of the two sites interacting. Because the reactive sites are typically only a few percent of the total surface area, any forces that direct oriented collisions dramatically enhance the reaction rate. The details of how the physics of the interactions are treated are important, but do not radically affect the computed enhancement of rates. This factor is the dominant term irrespective of the detailed nature of the computed interaction potential in the simulation.

NORTHRUP: What Ray Salemme is saying is true. We've done some preliminary calculations in our studies of cytochrome-like proteins reacting with a charged partner, and we've used a dipole moment corresponding to horse cytochrome. We find if you restrict the electron transfer region on cytochrome down to a 10° patch size, you can lower the rate down to 6% of the case where the whole surface of cytochrome is reactive. But when you put a dipole moment on, which steers the species into productive orientation on the surface, the dipole on cytochrome raises the rate of electron transfer back to about six times what it would be if those forces were absent. We see the same kind of steering mechanism Stuart Allison mentions, seeming to compensate for the fact that you have strong stereochemical constraints to the reactions.

SHARNOFF: The rate constant discussion has really focused on charge-induced charge effects . One could anticipate that a SO molecule approaching the SOD surface would induce its own localized charge distributions. One might regard the superoxide dismutase from the same conceptual standpoint as was addresed by Gary Ackers, namely one of local perturbations. In this case the perturbations are caused not by mutation but some other physical effect. Then, of course, the question arises as to whether there is any cooperativity involved in the structurally induced multipole in the protein that comes from the redistribution of charge by the approaching SO molecule.

ALLISON: I think you could put polarizability into the simulation, but I'm not sure how you would design it.

SHARNOFF: Normally, in referring to a dielectric constant one is referring to macroscopic behavior. You have to use an individual polarizability that in the long run would have to be referenced to the tertiary structure of the protein as well.

PARSEGIAN: I want to pick up a point Victor Bloomfield made about time scales. There is a history of recognition of force fields in aerosols and colloidal systems in solving many problems analytically where particles diffuse, and stick or don't stick. How do your methods supplement or reduce to those analytic systems? How about calibrating against the analytic solutions that have been in the literature for thirty years?

ALLISON: We've done that. Before we try to apply it to something as complicated as superoxide dismutase, we simulate known analytic problems to test the simulations. A good example is a uniformly reactive sphere with a charge embedded in it or a Solc-Stockmayer model where there are no direct forces but one molecule has a reactive path and the other is uniformly reactive.

POLLARD: I doubt that we are going to come back to diffusion, so I would like to add a worrisome note at this point. Most people who think about macromolecular assembly reactions assume that the subunit molecules are free to diffuse, but this may not actually be the case in some systems. Sato, Schwarz and I (Sato et al. 1985. *J. Biol. Chem.* 260:8585–8592) recently reported that solutions of actin molecules at low concentrations (1 mg/ml) in buffers where no filaments form are a viscoelastic solid. Thus at least part of the molecules must form some sort of a continuous network, even though they are a homogeneous population of monomers, judging from hydrodynamic measurements. This discrepancy may be due to two factors. First, standard hydrodynamic methods involve enough mechanical shearing to break the weak bonds between these molecules. Second, formation of these solids takes a long time, 10 or more hours. Subsequently, we have found that profilin and tubulin form viscoelastic solids. Solutions of cytochrome C are Newtonian fluids. Ovalbumin forms a weak viscoelastic liquid. Consequently, it is worth considering the possibility that nonfilamentous proteins may contribute to the mechanical properties of the cytoplasm. Also, one must be aware that at equilibrium some protein molecules may not diffuse as freely as suggested by hydrodynamic methods. This could influence macromolecular assembly processes that are usually thought to involve some diffusion-limited reactions of the subunits.