Substrate steering by electrostatic fields of enzymes: visualization by computer graphics

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The rates of reactions catalysed by certain enzymes are increased by electrostatic effects that steer substrate molecules towards the active sites of the enzyme. This phenomenon can be studied by using Brownian dynamics simulation to generate and analyse diffusional trajectories of substrates in the field of an enzyme. The paper demonstrates that computer graphics can be used to show how electrical fields influence the spatial dependence of substrate flux as calculated by Brownian dynamics. Because of the statistical noise associated with typical simulation runs, filtering techniques are helpful in determining systematic trends in the graphic displays.

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Electrostatic interactions between reactant molecules influence many biomolecular reactions[1]. One particularly interesting case is the reaction between the enzyme superoxide dismutase (SOD) and the substrate superoxide (O_2^-). It has been postulated that the detailed charge distribution of SOD may increase the reaction rate by steering O_2^- diffusion into the two active sites of the dimeric enzyme[2,3]. Recently, Allison *et al*[4] have shown that the rate constant of the SOD–O_2^- system is increased by substrate steering in the noncentrosymmetric electrical field of the enzyme. This paper describes computer graphics methods for visualizing the steering of O_2^- towards the active sites of SOD.

Physically, the most appropriate quantity to display in a spatial analysis of steering effects is the mean flux of substrate at different points around the enzyme. To obtain this flux, a set of diffusional trajectories for substrate molecules is computed by means of the Brownian dynamics simulation method[5]. This method is used because of its generality, a characteristic that enables it to model a system of arbitrary structural complexity. Also, inter- and intramolecular forces and hydrodynamic interactions can all be taken into account using this method. By analysing the diffusional trajectories of O_2^- relative to SOD, the flux of O_2^- (i.e. the product of average velocity and the probability density of O_2^- at each point) is computed. This data is then used to illustrate the steering of O_2^- due to the charge distribution of SOD.

In the next section, the Brownian dynamics simulation method is briefly reviewed, and the method of computing the flux of O_2^- is described. Also, the means of displaying the flux data on an Adage 3000 colour graphics system are discussed. Following presentation and discussion of the results, some concluding remarks and suggestions for further work are made.

METHODS

Brownian dynamics simulation method

The representative diffusional trajectories of O_2^- relative to SOD are computed using the Ermak and McCammon[6] algorithm for Brownian dynamics. The formula for the trajectory propagation is given by

$$\mathbf{r}(t + \Delta t) = \mathbf{r}(t) + (k_B T)^{-1} D_{\text{rel}} \Delta t\, \mathbf{F}(t) + \mathbf{S} \qquad (1)$$

where: $\mathbf{r}(t + \Delta t)$ and $\mathbf{r}(t)$ are the position vectors of O_2^- relative to the centre of SOD at times $t + \Delta t$ and t, respectively; $k_B T$ is the product of the Boltzmann constant and absolute temperature; D_{rel} is the diffusion coefficient of O_2^- relative to SOD; Δt is the time step; $\mathbf{F}(t)$ is the electrostatic force on O_2^- at time t; and $\mathbf{S}$ is a vector of Gaussianly distributed random numbers, which represent the stochastic displacements of O_2^- due to collisions with solvent molecules. $\mathbf{S}$ is computed using GGNML of the IMSL subroutine library[7]. The vector components of $\mathbf{S}$ have zero mean and a variance–covariance of

$$\langle S_i S_j \rangle = 2 D_{\text{rel}}\, \delta_{ij} \Delta t \qquad (2)$$

where δ_{ij} is the Kronecker delta function. The relative diffusion constant[4] $D_{\text{rel}} = k_B T(a_1 + a_2)/4\pi\eta a_1 a_2$, where η is the solvent viscosity, and a_1 and a_2 are the hydrodynamic radii of O_2^- and SOD. The force $\mathbf{F}(t)$ is computed using the following formula[8]:

$$F(t) = -\frac{e^2}{\varepsilon}\sum_{j=1}^{N} q_j(\mathbf{r} - \mathbf{r}_j)/|\mathbf{r} - \mathbf{r}_j|^3 \quad (3)$$

where q_j and r_j are, respectively, the magnitude and position of charge j on SOD, and N is the number of charges on SOD. In the present study, the dielectric constant has been taken to be 78 throughout the system. Additional details concerning this model are given elsewhere[4,9].

During the calculation of a trajectory, SOD is held fixed at the origin, and the relative motion of O_2^- is monitored. The time step used here is $\Delta t = 2$ ps. The trajectories are initiated with a uniform probability on a sphere of radius $r = b = 50$ Å. These initial positions were generated randomly using the GGSPH subroutine of the IMSL library[7]. Additional details concerning random numbers generated by GGSPH are discussed in Reference 10.

The SOD dimer is represented as a sphere of radius 28.5Å. The two active sites of SOD are represented as spherical caps, each of which makes an azimuthal angle of 10° with respect to an axis running through the centre of the dimer (see Figure 2 of Reference 4). This axis defines the y axis, with the centre of the enzyme acting as the origin for the right-handed coordinate system used in the present study. The superoxide molecule is represented as a sphere of radius 1.5Å, so that the collision radius with the enzyme is 30Å. The trajectories are terminated either when a collision occurs on a reactive surface (absorption boundary) or when $r \geqslant q$, where $q = 60$Å is the radius of a truncation sphere and is larger than b. When a collision occurs on a nonreactive surface (reflecting boundary) of the enzyme, that particular dynamic step is ignored, but the trajectory propagation time is incremented. A new step is then taken from the previous position with a different random displacement.

Calculation of flux

In order to observe the flux distribution of O_2^- close to the surface of SOD, a cubic lattice is placed around SOD, with a range of -51Å to 51Å on each axis. The lattice resolution is 2Å on each axis. Thus, the diffusion space is decomposed into cubic volume elements (voxels) of dimensions 2Å × 2Å × 2Å. Accumulator arrays for O_2^- number density and velocity are defined as ACCNUM(i, j, k) and ACCVEL(n, i, j, k); i, j and k range from -25 to $+25$ and identify the voxels, while n ranges from 1 to 3 corresponding to the x, y and z components of the velocity accumulator array. During a trajectory computation, if the O_2^- is in a particular voxel with indices i, j, k, ACCNUM(i, j, k) is increased by 1. The velocity vector defined as $[\mathbf{r}(t + \Delta t) - \mathbf{r}(t)]/\Delta t$ is computed, and the x, y and z components of this vector are added to the corresponding components of ACCVEL. The accumulation is done over a large number of trajectories. In the present study, 60 000 trajectories have been computed for each of several models of the charge distribution in SOD. This procedure normally needs about 6 h of CPU time on an AS/9000N computer, which is about ten times faster than a VAX 11/785 computer.

The above data is transferred to a VAX 11/785, which is the host for an Adage 3000 colour graphics system, for further processing and display. The average velocity components and the number density at a voxel i, j, k are obtained by the following equations:

$$\text{AVEVEL}(n, i, j, k) = \text{ACCVEL}(n, i, j, k)/\text{ACCNUM}(i, j, k) \quad (4)$$

$$\text{DEN}(i, j, k) = \text{ACCNUM}(i, j, k)/\sum_{i,j,k} \text{ACCNUM}(i, j, k) \quad (5)$$

Finally, the average velocity components at a voxel i, j, k are multiplied by the number density at voxel i, j, k to obtain the flux components at i, j, k.

Processing of the flux data

The flux of O_2^- around a given model of SOD was obtained by computing 60 000 trajectories of O_2^- relative to the enzyme. However, in order to get good statistics in each voxel, one would need to compute a much larger number of trajectories. Rather than do this (which is computationally expensive), image-processing techniques have been used to reduce the noise in the flux data prior to graphic display. The approach used here makes use of the fact that the correlation length of the flux is larger than the distance between voxels. Thus, averaging the flux values in a local neighbourhood helps to reduce the noise[11]. Additional details regarding noise removal in digital images are given elsewhere[11–13]. In the present study, a 3D filter has been used; the flux values in a 3 × 3 × 3 neighbourhood are averaged with equal weight, and the flux for the central voxel is replaced by this local average. By repeatedly applying the filter to all voxels, the neighbourhood in which the flux values are averaged increases, and the weight given for a voxel in the neighbourhood will depend on its distance from the central voxel[11]. In the present study, it has been observed that applying the filter two to four times gives reasonable results for the models considered.

As mentioned earlier, during the O_2^- trajectory computation, the trajectories are terminated when a collision occurs at the active sites of the enzyme. If the collision occurs on a nonreactive surface of the enzyme, that particular dynamic step is ignored and the trajectory propagation time is incremented. This leads to a hole, the volume of which is equal to the collision volume, in the 3D flux of O_2^- around SOD. When filtering the data, those voxels that belong to the hole have not been considered for averaging purposes.

Display of flux data

The flux components at a voxel are very small real numbers ($O(10^{-7})$ in units of Å ps^{-1}). By multiplying by a constant scale factor, these components are converted to integers. This 3D data is divided into several cross-sections. In the present study, cross-sections perpendicular to the z axis have been taken, and the flux at each voxel has been displayed as a 3D arrow in the direction of the flux; the length of the arrow is proportional to the magnitude of the flux at the voxel. White has been used to represent the head of the flux vector and red to represent the tail. Here, only the data from the central cross-section is presented, since this contains much of the information necessary for visualizing the steering of O_2^- towards the active sites of SOD.

RESULTS AND DISCUSSION

The substrate-steering phenomenon for SOD can be illustrated qualitatively by considering two different models of the charge distribution in the enzyme. The first model has a single charge of -4 at the centre of the enzyme. The model corresponds to the monopole term of the actual charge distribution and leads to a centrosymmetrical electrical field that cannot steer substrate into the active sites (see Colour Plate 1a). The second model has five charges embedded in the enzyme to reproduce the monopole, dipole and quadrupole components of the charge distribution in SOD; the possibility of substrate steering is shown clearly in Colour Plate 1b. The details of the charge distributions are given in Reference 4.

Colour Plates 2 and 3 display the flux of O_2^- within 50Å of the centre of SOD. In all the figures, only the central cross-section data has been presented. The green circle represents the collision sphere, and the spherical cap enclosed by a purple line represents the active site of SOD. The unfiltered flux is displayed in Colour Plate 2. Here, the scale factor used to multiply the flux data for display purposes is 1.5×10^8. As can be seen from the pictures, the data is very noisy, and it is difficult to draw conclusions concerning steering effects. To improve the signal-to-noise ratio, a filter was used as described in the methods section. Colour Plate 3 displays the flux of O_2^- around the two SOD models after filtering the flux data three times in a $3 \times 3 \times 3$ neighbourhood. Here, the scale factor used to multiply the flux data for display purposes is 1.2×10^9. The steering effects are clearly more apparent in the filtered data than in the raw data. In particular, comparison of the two parts of Colour Plate 3 shows that the noncentrosymmetrical components of the electrical field favour inward and outward flow of substrate near the active sites and the equatorial regions of the enzyme, respectively.

CONCLUDING REMARKS

Computer graphics has been shown to be helpful in demonstrating how the charge distribution of an enzyme can increase the diffusion of substrate molecules to the active sites of the enzyme. It has also been shown that image-processing filtering techniques can improve the clarity of diffusional patterns in the presence of the statistical noise that inevitably results from finite sampling in substrate-diffusion simulations. In principle, such graphic analysis may be helpful in suggesting where an enzyme could be modified to optimize substrate steering. The work described here could be extended in a variety of ways. In the present study, fairly simple models have been used for the charge distribution of the enzyme. However, it is possible to consider the detailed charge distribution of SOD using the lattice method[8]. Also, the Colour Plates have displayed only planar projections of central cross-section data for the flux of O_2^- around SOD. To visualize the 3D data, one can display stereo pairs of the flux vectors. The visualization can be further enhanced by generating stereo pairs from different viewing angles around SOD and displaying them in succession at short intervals to produce the effect of animation. Research along these lines is in progress.

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REFERENCES

1 **McCammon, J A and Harvey, S C** *Dynamics of proteins and nucleic acids* Cambridge University Press, UK (in press)
2 **Getzoff, E D et al.** *Nature* Vol 306 (1983) pp 287–290
3 **Cudd, A and Fridovich, I** *J. Biolog. Chem.* Vol 257 (1982) pp 11443–11447
4 **Allison, S A, Ganti, G and McCammon, J A** *Biopolym.* Vol 24 (1985) pp 1323–1336
5 **Northrup, S H, Allison, S A and McCammon, J A** *J. Chem. Phys.* Vol 80 (1984) pp 1517–1524
6 **Ermak, D L and McCammon, J A** *J. Chem. Phys.* Vol 69 (1978) pp 1352–1360
7 *IMSL Library 7 Reference Manual* IMSL International and Statistical Libraries, USA (1975)
8 **Ganti, G, McCammon, J A and Allison, S A** *J. Phys. Chem.* Vol 89 (1985) pp 3899–3902
9 **Allison, S A and McCammon, J A** *J. Phys. Chem.* Vol 89 (1985) pp 1072–1074
10 **Marsaglia, G** *Ann. Math. Stat.* Vol 3 (1972) pp 645–646
11 **Rosenfeld, A and Kak, A C** *Digital picture processing Vol 1* Academic Press, USA (1982)
12 **Davis, L S and Rosenfeld, A** *IEEE Trans. Syst., Man & Cybern.* Vol 8 (1978) pp 705–710
13 **Dhawan, A P, Buelloni, G and Gordon, R** *IEEE Trans. Med. Image.* Vol MI-5 (1986) pp 8–15