Biophysical Journal Volume 65 September 1993 1155-1161

Macromolecular Diffusion in Crowded Solutions

Jining Han and Judith Herzfeld
Department of Chemistry, Brandeis University, Waltham, Massachusetts 02254 USA

ABSTRACT The effects of crowding on the self or tracer diffusion of macromolecules in concentrated solutions is an important but difficult problem, for which, so far, there has been no rigorous treatment. Muramatsu and Minton suggested a simple model to calculate the diffusion coefficient of a hard sphere among other hard spheres. In this treatment, scaled particle theory is used to evaluate the probability that the target volume for a step in a random walk is free of any macromolecules. We have improved this approach by using a more appropriate target volume which also allows the calculation to be extended to the diffusion of a hard sphere among hard spherocylinders. We conclude that, to the extent that proteins can be approximated as hard particles, the hindrance of globular proteins by other proteins is reduced when the background proteins aggregate (the more so the greater the decrease in particle surface area), the hindrance due to rod-shaped background particles is reduced slightly if the rod-like particles are aligned, and the anisotropy of the diffusion of soluble proteins among cytoskeletal proteins will normally be small.

1. INTRODUCTION

The diffusion of macromolecules inside cells is hindered by the very high concentrations of cellular proteins (typically 20–30 vol%). The cellular proteins occur in various shapes and sizes, including aggregates which, in the case of cytoskeletal fibers, may be very long. In order to understand cell dynamics, it is important to improve our understanding of the diffusion of macromolecules in this crowded environment.

The problem of a large particle diffusing in solution was studied by Einstein (1956) at infinite dilution. In this limit, interactions between large particles can be neglected and the interaction between the large particle and the solvent particles can be replaced by a randomly fluctuating force. The problem can then be solved by using the Langevin equation or a Focker-Planck-type master equation, which in this case reduces to the usual Fick diffusion equation. The equations can be solved readily in this case since we are only dealing with a one-body problem.

If the concentration of the large particles is significant, one has to consider the interactions between the large particles, a complicated many-body problem. The usual approach is to use the Smoluchowski equation, generalizing the single particle diffusion equation to $3N$ dimensions, where N is the number of large particles. Although the general solution to the Smoluchowski equation is still not known, there have been several attempts (Marquesee and Deutch, 1980; Tough, 1982; Hanna et al., 1982) to solve the Smoluchowski equation to first order in the concentration of the large particles. This gives the concentration dependence of the self-diffusion coefficient in the dilute limit. The result is unambiguous if one considers only the direct interaction between large particles. However, it is well known that large particles also interact at long range, through the solvent molecules. This hydrodynamic interaction does not yet have an accurate expression; one approximation of hydrodynamic interaction gives a contribution comparable to that of the excluded volume, and another approximation gives a smaller contribution (Hanna et al., 1982).

Under crowded conditions, where short-range interactions dominate but a rigorous solution to Smoluchowski equation proves to be difficult, we explore alternative routes. In equilibrium statistical mechanics, one also often faces many-body problems. However, if the system under study is not in the critical region, mean field theory provides a good approximation to the many-body problem. Mean field theory reduces the many-body problem to a one-body problem by considering the motion of a single particle under some averaged interaction exerted by all other particles. In this paper we use a similar idea to study the self-diffusion of one large particle among other large particles, in addition to the small solvent particles. This method was first suggested by Muramatsu and Minton (1988) when they studied the tracer diffusion of one type of protein in a solution of another type of protein. Scaled particle theory (Reiss et al., 1959) is used by Muramatsu and Minton to evaluate the probability of success of a step in a random walk. We also use scaled particle theory for the same purpose but with a more appropriate target volume. With this formulation, we are able to calculate the diffusion tensor in the case of anisotropic diffusion of a hard sphere among hard rods. The resulting expression depends on a single unknown model parameter that is specific to the tracer particle and the solvent properties but independent of the properties of the other solute species. We can obtain this parameter easily by fitting the existing experimental data. With the parameter obtained this way, we can predict the diffusion tensor for the same tracer particle among other solute particles with varying axial ratios and orientations, in the same solvent.

Received for publication 12 April 1993 and in final form 9 June 1993.
Address reprint requests to Dr. Judith Herzfeld, Department of Chemistry, P. O. Box 9110, Brandeis University, Waltham, MA 02254-9110.

0006-3495/93/09/1155/07 $2.00

The rest of the paper is arranged as follows. In the next section, we will use scaled particle theory to derive the diffusion coefficient of a hard sphere among hard spherocylinders with arbitrary size and orientation distribution. In section 3, we apply our general result to the special case of a hard sphere diffusing among other hard spheres and fit experimental self-diffusion data with a single adjustable model parameter. The parameter thus obtained is used to estimate the anisotropy of the diffusion of the same hard sphere among monodisperse hard spherocylinders. In section 4 we draw our conclusions.

2. FORMULATION

Following Muramatsu and Minton (1988), let us consider initially one large particle diffusing among small solvent particles. The motion of the large particle is treated as a three-dimensional random walk with a fixed effective step size Δr. The self-diffusion coefficient is then given by

$$D_0 = \frac{(\Delta r)^2}{6\Delta t}, \tag{1}$$

with Δt denoting the time for each step in the random walk. If we now introduce some other large particles (i.e., consider the problem of one hard sphere diffusing at finite concentrations of macromolecules), the diffusing particle may run into these macromolecules in some steps. Whenever this happens, the motion is hindered. For simplicity, we will suppose that the motion of the particle is stopped completely. In other words, the random walk step is not realized in such a case and the particle stays at the same place after Δt. The reduced diffusion coefficient thus depends on the probability that the diffusing particle does not run into other particles in a single step of the random walk. We therefore calculate the probability of an unobstructed step for a hard sphere of radius R. In one step of the walk, this particle will sweep a spherocylinder with radius R and cylinder length Δr. If we exclude the volume of the sphere itself from this spherocylinder, the remaining volume will be referred to as the target volume (see Fig. 1). We will use scaled particle theory to evaluate the probability that this target volume is free of any particles.

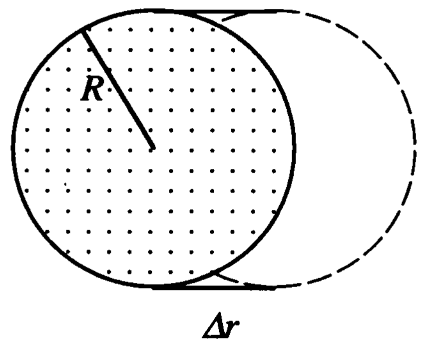

FIGURE 1 Target volume for a step in a random walk of a spherical particle.

Since we are dealing with spherocylindrical volumes, we will follow the formulation of scaled particle theory by Cotter (1977). According to this theory, the reversible work that has to be done in order to insert a hard spherocylinder, with orientation ω_t, radius r_t, and cylindrical length l_t, into a system of hard spherocylinders, with number concentration c and normalized radius, length, and orientation distribution $f(r, l, \omega)$, is given by

$$\begin{aligned}\beta W(\Omega_t, r_t, l_t) &= \beta p\left(\frac{4}{3}\pi r_t^3 + \pi r_t^2 l_t\right) - \ln(1 - v_p) \\ &\quad + \frac{c}{1 - v_p}\langle 4\pi r_t r(r_t + r) + 2\pi r_t r(l_t + l) \\ &\qquad + \pi(l_t r^2 + l r_t^2) + 2 l_t l(r_t + r)\sin\theta(\Omega, \Omega_t)\rangle \\ &\quad + \frac{c^2}{(1 - v_p)^2}\langle 2\pi r(l + 2r)\rangle \\ &\quad \times \langle r_t r(2\pi r_t r + \pi l_t r + \pi l r_t + 2 l_t l \sin\theta(\Omega, \Omega_t))\rangle, \end{aligned} \tag{2}$$

where p is the pressure, v_p is the volume fraction of the polydisperse hard spherocylinders, $\theta(\Omega, \Omega_t)$ is the angle between Ω and Ω_t, and $\langle\ \rangle$ denotes the average over the distribution $f(r, l, \Omega)$. The pressure given by scaled particle theory is

$$\beta p = \frac{c}{1 - v_p} + \frac{1}{2}B\frac{c^2}{(1 - v_p)^2} + \frac{2}{3}C\frac{c^3}{(1 - v_p)^3}, \tag{3}$$

where

$$\begin{aligned} B &= \langle\langle 4\pi r r'(r + r') + 2\pi r r'(l + l') + \pi(l r'^2 + l' r^2) \\ &\qquad + 2 l l'(r + r')\sin\theta(\Omega, \Omega')\rangle\rangle \\ C &= \langle 2\pi r(l + 2r)\rangle\langle\langle r r'(2\pi r r' + \pi l r' + \pi l' r \\ &\qquad + 2 l l' \sin\theta(\Omega, \Omega'))\rangle\rangle \end{aligned}$$

with $\langle\langle\ \rangle\rangle$ denoting the average over both primed and unprimed variables. We also need the reversible work for inserting a hard sphere, which is obtained easily by setting l_t in Eq. 2 to be zero, with a result that is independent of Ω_t. It follows that the conditional probability that the target volume for movement of a sphere of radius r_t, in direction Ω_t, by a distance l_t is free of any particles, is given by

$$P(\Omega_t, r_t, l_t) = \frac{\exp[-\beta W(\Omega_t, r_t, l_t)]}{\exp[-\beta W(0, r_t, 0)]}. \tag{4}$$

To use this expression in our diffusion calculation, one should identify $r_t = R$, the radius of the diffusing particle, and $l_t = \Delta r$, the random walk step size. If the solution is isotropic, then $P(\Omega_t, R, \Delta r)$ is independent of Ω_t, and the reduced diffusion coefficient is simply

$$D = D_0 P(0, R, \Delta r). \tag{5}$$

In general the diffusion coefficient tensor is given by

$$\begin{aligned} D_{ij} &= \frac{\langle \Delta r_i \Delta r_j P(\Omega_t, R, \Delta r)\rangle_{\Omega_t}}{2\Delta t} \\ &= \frac{(\Delta r)^2}{2\Delta t} \langle (\hat{\Omega}_t \cdot \hat{\imath})(\hat{\Omega}_t \cdot \hat{\jmath}) P(\Omega_t, R, \Delta r)\rangle_{\Omega_t}, \end{aligned} \tag{6}$$

where the average is taken over the direction Ω_t of the random walk. Suppose that the solution is nematic with its director designated as the z-axis of the coordinate system. In this coordinate system the diffusion coefficient tensor is diagonal and its three diagonal components are

$$D_z = 3D_0 \langle (\hat{\Omega}_t \cdot \hat{z})^2 P(\Omega_t, R, \Delta r)\rangle_{\Omega_t} \tag{7}$$

and

$$\begin{aligned} D_x = D_y &= 3D_0 \langle (\hat{\Omega}_t \cdot \hat{x})^2 P(\Omega_t, R, \Delta r)\rangle_{\Omega_t} \\ &= 3D_0 \langle (\hat{\Omega}_t \cdot \hat{y})^2 P(\Omega_t, R, \Delta r)\rangle_{\Omega_t} \\ &= {}^3\!/\!_2 D_0 \langle (1 - (\hat{\Omega}_t \cdot \hat{z})^2) P(\Omega_t, R, \Delta r)\rangle_{\Omega_t}. \end{aligned} \tag{8}$$

In the following we will use $D_{\|}$ to denote D_z, and $D_{\perp}$ to denote D_x and D_y. Two relatively simple limiting cases will be instructive.

Isotropic monodisperse rods

For perfectly isotropically oriented spherocylinders of radius ρ and cylinder length λ,

$$D = D_0 \exp\left[-\left(\frac{\Delta r}{R}\right)\xi\right], \tag{9}$$

where ξ is a monotonic dimensionless function of concentration

$$\xi = a_1 \frac{v_p}{(1 - v_p)} + a_2 \frac{v_p^2}{(1 - v_p)^2} + a_3 \frac{v_p^3}{(1 - v_p)^3} \tag{10}$$

with coefficients

$$a_1 = \frac{\left[\left(\frac{R}{\rho}\right)\left(\frac{R}{\rho} + 1\right)^2 + \frac{1}{2}\left(\frac{\lambda}{\rho}\right)\left(\frac{R}{\rho}\right)\left(\frac{R}{\rho} + 1\right)\right]}{\left(\frac{\lambda}{\rho} + \frac{4}{3}\right)}, \tag{11a}$$

$$a_2 = \frac{\left[\frac{1}{2}\left(\frac{\lambda}{\rho} + 2\right)\left(\frac{\lambda}{\rho} + 4\right)\left(\frac{R}{\rho}\right)^3 + \left(\frac{\lambda}{\rho} + 2\right)^2\left(\frac{R}{\rho}\right)^2\right]}{\left(\frac{\lambda}{\rho} + \frac{4}{3}\right)^2}, \tag{11b}$$

and

$$a_3 = \frac{2}{3}\left(\frac{\lambda}{\rho} + 2\right)^3\left(\frac{R}{\rho}\right)^3 \Big/ \left(\frac{\lambda}{\rho} + \frac{4}{3}\right)^3. \tag{11c}$$

Eq. 9 can be compared with the stretched exponential $D = D_0 \exp(-\alpha v_p^{\beta} R^{\gamma})$ commonly used to describe tracer diffusion in polymer solutions. Since, as we shall show below, $\Delta r/R$ is a constant, Eq. 9, in the limit of small v_p and small R/ρ, corresponds to a stretched exponential with $\beta = 1$ and $\gamma = 1$. More generally, Eq. 9 corresponds to a product of stretched exponentials, one of which would have the above form ($\beta = 1$, $\gamma = 1$) and the rest of which would have larger integer values of β and γ.

Parallel monodisperse rods

The calculation for parallel spherocylinders gives parallel and perpendicular diffusion tensor components

$$D_{\|} = D_0 \exp\left[-\left(\frac{\Delta r}{R}\right)\xi\right] \times \left\langle 3\cos^2\theta \exp\left[-\left(\frac{\Delta r}{R}\right)\zeta \sin\theta\right]\right\rangle \tag{12}$$

and

$$D_{\perp} = D_0 \exp\left[-\left(\frac{\Delta r}{R}\right)\xi\right] \times \left\langle \frac{3}{2}\sin^2\theta \exp\left[-\left(\frac{\Delta r}{R}\right)\zeta \sin\theta\right]\right\rangle \tag{13}$$

where ξ is given by Eq. 10 with coefficients

$$a_1 = \left(\frac{R}{\rho}\right)\left(\frac{R}{\rho} + 1\right)^2 \Big/ (\frac{\lambda}{\rho} + \frac{4}{3}, \tag{14a}$$

$$a_2 = \frac{\left[3\left(\frac{\lambda}{\rho} + \frac{4}{3}\right)\left(\frac{R}{\rho}\right)^3 + 2\left(\frac{\lambda}{\rho} + 2\right)\left(\frac{R}{\rho}\right)^2\right]}{\left(\frac{\lambda}{\rho} + \frac{4}{3}\right)^2}, \tag{14b}$$

and

$$a_3 = \frac{8}{3}\left(\frac{\lambda}{\rho} + 1\right)\left(\frac{\lambda}{\rho} + 2\right)\left(\frac{R}{\rho}\right)^3 \Big/ \left(\frac{\lambda}{\rho} + \frac{4}{3}\right)^3, \tag{14c}$$

and ζ is another monotonic dimensionless function of concentration

$$\zeta = a_1' \frac{v_p}{(1 - v_p)} + a_2' \frac{v_p^2}{(1 - v_p)^2} \tag{15}$$

with coefficients

$$a_1' = \frac{2}{\pi}\left(\frac{\lambda}{\rho}\right)\left(\frac{R}{\rho}\right)\left(\frac{R}{\rho} + 1\right) \Big/ \left(\frac{\lambda}{\rho} + \frac{4}{3}\right) \tag{16a}$$

and

$$a_2' = \frac{4}{\pi}\left(\frac{\lambda}{\rho}\right)\left(\frac{\lambda}{\rho} + 2\right)\left(\frac{R}{\rho}\right)^2 \Big/ \left(\frac{\lambda}{\rho} + \frac{4}{3}\right)^2. \tag{16b}$$

The isotropic average of the diffusion coefficient is given by

$$\begin{aligned} D &= \tfrac{1}{3}(D_{\|} + 2D_{\perp}) \\ &= D_0 \exp\left[-\left(\frac{\Delta r}{R}\right)\xi\right]\left\langle \exp\left[-\left(\frac{\Delta r}{R}\right)\zeta \sin\theta\right]\right\rangle, \end{aligned} \tag{17}$$

and a measure of the anisotropy of the diffusion tensor is given by

$$D_{\|}/D_{\perp} = \frac{2\left\langle \cos^2\theta \exp\left[-\left(\frac{\Delta r}{R}\right)\zeta \sin\theta\right]\right\rangle}{\left\langle \sin^2\theta \exp\left[-\left(\frac{\Delta r}{R}\right)\zeta \sin\theta\right]\right\rangle}. \tag{18}$$

Notice that in the limiting case of spherical background particles ($\lambda \rightarrow 0$), for which isotropic and parallel orientations are meaningless, Eqs. 9, 12, 13, and 17 give identical results, as expected.

3. RESULTS AND DISCUSSION

Self-diffusion of globular proteins

It would be desirable to compare the general results of the above theory with experimental data. However, since we are unaware of any experimental measurements of anisotropic tracer diffusion, we only analyze data for self-diffusion of globular proteins ($\lambda = 0$ and $\rho = R$). In this case, Eqs. 9–11 or 12–17 reduce to

$$\ln D = \ln D_0 - \left(\frac{\Delta r}{R}\right)\xi \tag{19}$$

with

$$\xi = 3\frac{v_p}{1 - v_p} + \frac{9}{2}\frac{v_p^2}{(1 - v_p)^2} + \frac{9}{4}\frac{v_p^3}{(1 - v_p)^3}. \tag{20}$$

This result is used to analyze experimental data in Fig. 2. One can see that our prediction of a linear relationship between $\ln D$ and concentration variable ξ is in good agreement with the experimental data. The values of $\Delta r/R$ obtained from the slope of the least-square fit are listed in Table 1. We see that $\Delta r/R$ is roughly between 0.5 and 1.0. To see that this range is reasonable, consider the dilute limit, i.e., $v_p \rightarrow 0$. In this limit, $\xi = 3v_p$ and

$$D = D_0\left[1 - 3\left(\frac{\Delta r}{R}\right)v_p\right]. \tag{21}$$

In the same dilute limit, the Smoluchowski equation gives

$$D = D_0(1 - \alpha v_p) \tag{22}$$

with $\alpha = 2$ for the direct hard core interaction, that is, the pure excluded volume effect (Hanna et al., 1982). Since Eq. 21 is also a result of the same interaction, Eqs. 21 and 22 should be comparable, and this comparison yields $\Delta r/R = 2/3$. This value, which corresponds to a target volume equal to one-half of the volume of the spherical tracer, is right in the range obtained by fitting the experimental data.

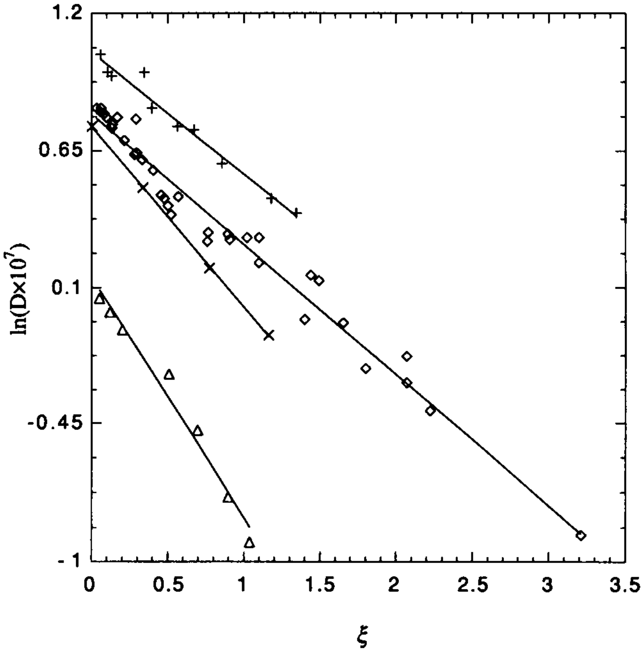

FIGURE 2 Experimental data for protein self diffusion (Gros, 1978): (◇) hemoglobin; (+) myoglobin; (△) invertebrate hemoglobin; (×) ovalbumin. The lines represent least-square fits of Eq. 19. The unit for D is cm^2/s. The concentration variable ξ, on the abscissa, is calculated according to Eq. 20 using 0.8 ml/g as the specific volume for each protein.

TABLE 1 The random walk step size obtained by fitting the experimental diffusion data for four different soluble globular proteins as shown in Fig. 2.

	M (daltons)	$\Delta r/R$	$\ln(D_0 \times 10^7)$
Myoglobin	17,000	0.49	1.04
Hemoglobin	65,000	0.52	0.799
Ovalbumin	43,500	0.73	0.749
Invertebrate hemoglobin	3,700,000	0.96	0.142

The molecular weight and the diffusion coefficient at infinite dilution are listed for each protein. The unit for D_0 is cm^2/s.

In their treatment, Muramatsu and Minton (1988) used a target volume expanding in three dimensions. Therefore, to describe the same data their step size $\Delta r'$ must be smaller than our step size Δr. Empirically we found that our Δr is roughly twice as large as the value of $\Delta r'$ obtained by Zimmerman and Minton (1993) using the equation of Muramatsu and Minton (1988). The quality of our fit is comparable to that of Zimmerman and Minton's (1993). However, Eq. 19 in this paper is considerably simpler than its counterpart in Muramatsu and Minton (1988), since the dependence of $\ln D$ on the unknown parameter Δr is cleanly separated in Eq. 19 from the dependence on the concentration of particles.

Tracer diffusion in an isotropic background

In the rest of the paper we will use the general result of section 2 and the value for $\Delta r/R = 2/3$ obtained from the Smoluchowski equation to make estimates of the diffusion of a hard sphere tracer particle among monodisperse hard spherocylinders. Fig. 3 shows the dependence of tracer diffusion on the volume fraction of isotropically oriented background particles of various sizes and shapes. In Fig. 3 *a*, the values of λ and ρ are chosen so that the volumes of the various types of background particles are all 16 times that of the tracer particle. Thus, although the axial ratios of the background particles vary, their number concentration, at a given solute volume fraction, v_p, is the same. We see that the more compact, spherical ($\lambda = 0$) background particles hinder tracer diffusion less than elongated background particles of the same volume. This suggests that particle surface area might be the critical variable. In Fig. 3 *b*, the values of λ and ρ are chosen so that the surface-to-volume ratios for the various types of background particles are all the same. Thus, although the axial ratios of the background particles vary, the particle surface area, at a given solute volume fraction, v_p, is the same. We see that these particles hinder tracer diffusion to a similar extent, and conclude that, to a first approximation, the diffusion rate is limited by the surface area of the obstacles. The bulk inside the particle surface, which is greatest for the spherical ($\lambda = 0$) particles, has a relatively small effect.

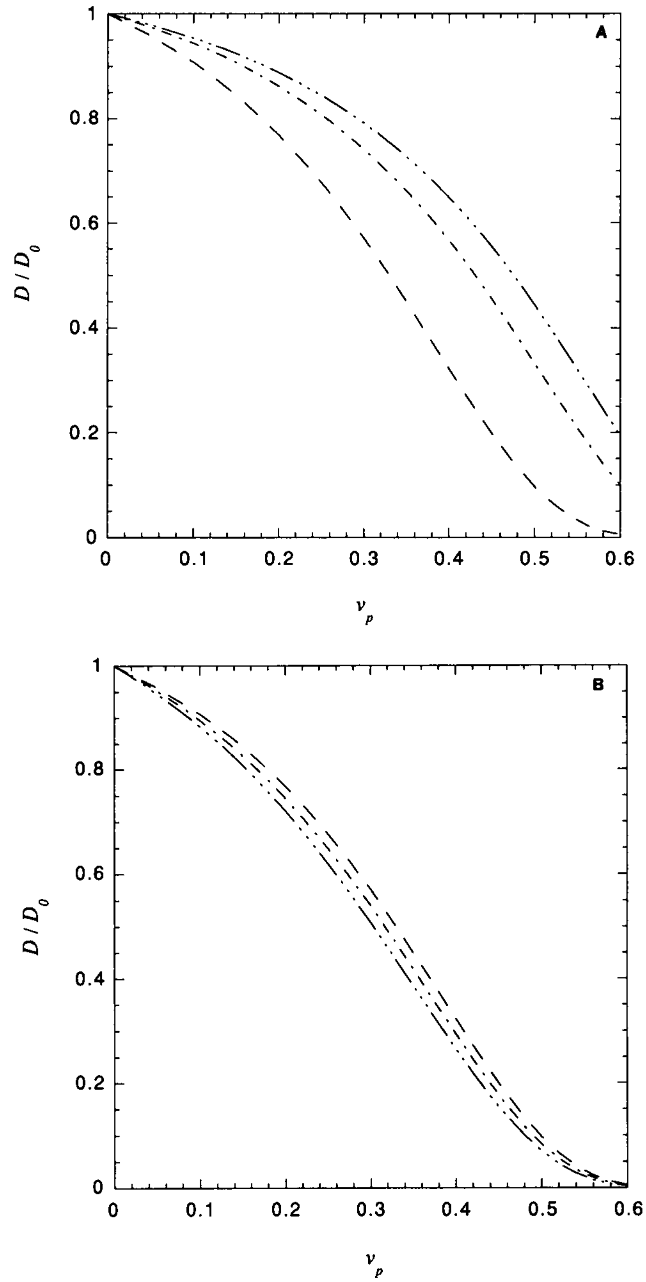

FIGURE 3 Theoretical prediction of the dependence on solute volume fraction, v_p, of the diffusion coefficient of a hard sphere among isotropic hard spherocylinders. (*A*) Spherocylinders of constant volume/particle but varying axial ratio: $\lambda/\rho = 20$, $\rho/R = 1$ (– – – –); $\lambda/\rho = 4$, $\rho/R = 4^{1/3}$ (– · – · – ·); $\lambda/\rho = 0$, $\rho/R = 16^{1/3}$ (– · · · – · · ·). (*B*) Spherocylinders of constant surface-to-volume ratio, but varying axial ratio: $\lambda/\rho = 20$, $\rho/R = 1$ (– – – –); $\lambda/\rho = 4$, $\rho/R = 12/11$ (– · – · – ·); $\lambda/\rho = 0$, $\rho/R = 16/11$ (– · · · – · · ·).

Tracer diffusion in an anisotropic background

An intended advantage of our approach is that it can presumably give an estimate of anisotropic diffusion. In order to carry out the calculation, one has to specify the orientational distribution function of the background particles, $f(\Omega)$. It is well known that scaled particle theory gives a good description of the system. In particular, according to this theory, the distribution function for monodisperse spherocylinders with radius ρ and cylinder length λ satisfies the following nonlinear integral equation:

$$\ln f(\Omega) + \chi + 4c\rho\lambda^2 \frac{1 - (\pi/3)c\rho^2\lambda}{(1 - v_p)^2} \int f(\Omega')\sin\theta(\Omega, \Omega')\, d\Omega' = 0, \quad (23)$$

where χ is the Lagrange multiplier for the normalization of the distribution $f(\Omega)$. This equation can be solved readily using the algorithm of Herzfeld et al. (1984). With this distribution function, we have calculated $D_{\parallel}/D_{\perp}$ using Eqs. 7 and 8. However, it is simpler and more instructive to discuss the two extreme cases of perfectly isotropic rods and perfectly parallel rods.

Eqs. 9–11 and 14–17 show that D is a monotonically increasing function of λ/ρ. Physically this means that when background particles aggregate to form rodlike particles, the hindrance is reduced, and the longer the rods, the smaller the hindrance. This effect, which is eventually saturated for long rods, is consistent with the decrease in particle surface area on aggregation. This dependence of D/D_0 on λ/ρ is demonstrated in Fig. 4 where D/D_0 is plotted versus v_p for three different axial ratios ($\lambda/\rho = 0$, 20, and 1000). For $\lambda/\rho > 0$, curves are plotted for both isotropic and parallel spherocylinders. We see that the curve for parallel spherocylinders is always above that for isotropic spherocylinders of the same axial ratio, indicating that the hindrance to diffusion is further reduced if the background particles are aligned.

D/D_0 also depends on the relative size of the tracer and the background species. Eqs. 11, 14, and 16 show that this dependence is strong, as illustrated in Fig. 5 *a*. If $R \ll \rho$, Δr is also small and the small tracer particle hardly runs into any

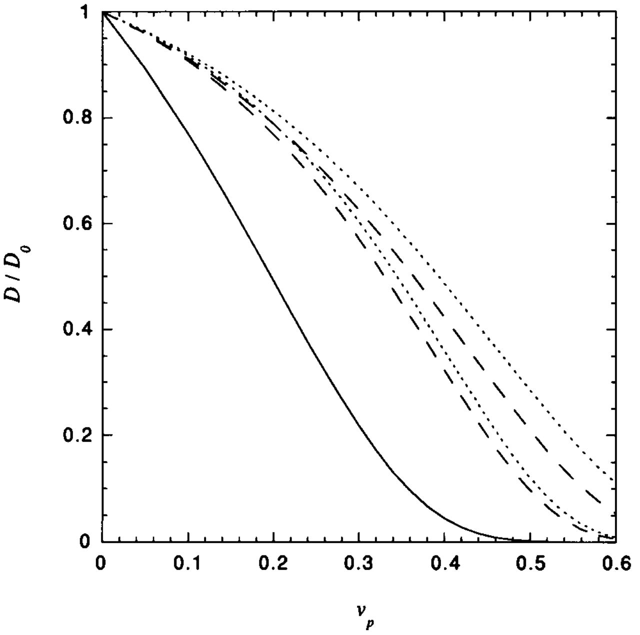

FIGURE 4 Theoretical prediction of the dependence on solute volume fraction, v_p, of the isotropic average of the diffusion coefficient of a hard sphere among hard spherocylinders of the same radius ($\rho = R$) and various axial ratios: $\lambda/\rho = 0$ (——); 20 (– – – –); 1000 (- - - - -). For the nonspherical background particles ($\lambda \neq 0$), the upper and the lower curves correspond to parallel and isotropic orientations, respectively.

background particles in a step of the random walk. Thus D/D_0 goes to 1 in this limit. If $\rho < R < \lambda$, the background particles can block the motion of the tracer particles effectively; we expect a large hindrance of tracer diffusion, and Eqs. 11, 14, and 16 are consistent with this intuition. If $R > \lambda$, these equations give a still smaller D/D_0. However, in this case our assumption that the tracer particle is stopped completely when it runs into a background particle is invalid. A small background particle certainly cannot stop a large tracer particle. Thus it is not surprising that our equations predict a far greater hindrance of colloidal particles among rodlike polymers than is found experimentally (Jamieson et al., 1982; Tracy and Pecora, 1992).

Finally we consider the anisotropy of tracer diffusion among parallel spherocylinders. We find that the dependence of $D_\parallel/D_\perp$ on λ/ρ is generally weak. Fig. 5 *b* shows $D_\parallel/D_\perp$ versus v_p for two values of λ/ρ (20 and 1000) and three values of R/ρ (0.5, 1, and 2). We see that for the same value of R/ρ, two very different values of λ/ρ produce very little difference in $D_\parallel/D_\perp$. However, the dependence of $D_\parallel/D_\perp$ on R/ρ is very strong. Fig. 5 *b* shows that for small R/ρ, $D_\parallel/D_\perp$ is close to 1. Intuitively this is because the small tracer particle has a small random walk step and zigzags around the larger background particles irrespective of their orientation. For $R/\rho = 1$, $D_\parallel/D_\perp$ is less than 2 for volume fractions up to 50%. The dependence of $D_\parallel/D_\perp$ on volume fraction is much stronger for $R/\rho = 2$, and the theory predicts a still greater $D_\parallel/D_\perp$ for still larger R/ρ.

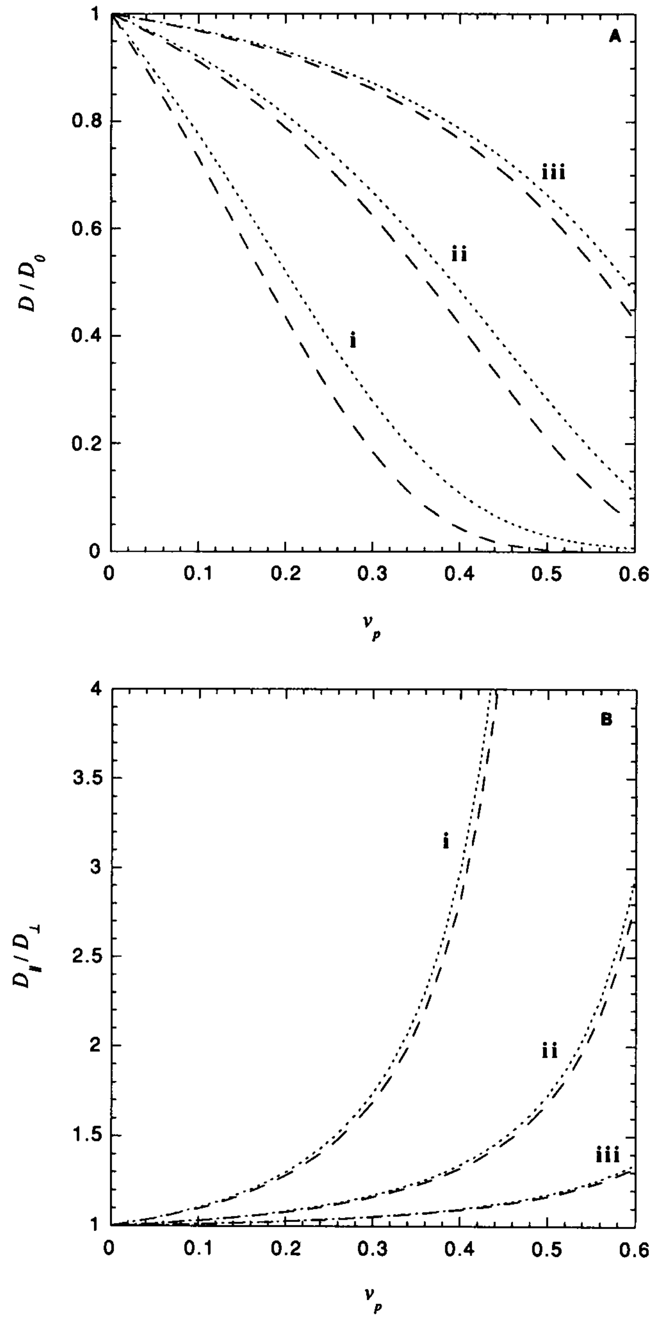

FIGURE 5 Theoretical prediction of the dependence on solute volume fraction, v_p, of (*A*) the isotropic average of the diffusion tensor, and (*B*) the anisotropy of the diffusion tensor, of a hard sphere among parallel hard spherocylinders of axial ratio: $\lambda/\rho = 20$ (– – – –), 1000 (- - - - -) for varying tracer size: $R/\rho = 2$ (i), 1 (ii), 0.5 (iii).

Other considerations

We see in Figs. 3–5 that, for globular proteins (as opposed to colloidal spheres), crowding (in the strict sense of excluded volume) significantly inhibits diffusion only at macromolecule concentrations approaching or exceeding those found in cells (usually 20–30 vol%). Effects at lower concentrations require long-range interactions between the particles, for example, due to hydrodynamic interactions or electrostatic repulsions. Such effects have been observed in the diffusion of bovine serum albumin in semidilute solutions of DNA (Wattenbarger et al., 1992) and the diffusion of size-fractionated Ficolls in semidilute solutions of F-actin (Hou et al., 1990). Hard-particle theories are sometimes adjusted to take long-range repulsions into account by defining an enlarged effective particle radius. In this case, the effective volume fraction is much larger than the hard core volume fraction, which can crudely explain the effects seen in semidilute solutions. The problem with this approach is that the appropriate effective particle radius decreases with increasing concentration, as particles are forced into the range of distances that they avoided at lower concentrations. Because of this collapse in effective particle size with increasing concentration, the dependence of the diffusion rate on concentration will be weaker than the high-order dependence we calculate for truly crowded solutions.

4. CONCLUSIONS

We have derived the diffusion tensor of a hard sphere in anisotropic solutions. Our prediction for the concentration dependence of the self-diffusion of a hard sphere is in good agreement with the existing experimental data for soluble proteins. Using a model parameter obtained by comparison with rigorous treatment of the dilute limit, we have made quantitative predictions for the diffusion of a hard sphere among monodisperse hard spherocylinders. The theory predicts that, under conditions where proteins can be approximated as hard particles (i.e., near the isoelectric pH of the background particles), the hindrance of globular proteins by other proteins at a given volume fraction is reduced when the background proteins are aggregated, and the hindrance is somewhat further reduced if rodlike aggregates are aligned. The anisotropy of the tracer diffusion is generally predicted to be weak, even if the solutions are very crowded (volume fractions up to 50%) and the background particles are perfectly aligned. Significant anisotropy is expected only when the diameter of the tracer particle is larger than that of the aligned background particles. Thus it is predicted that the diffusion of globular proteins in cells will generally be slower than in dilute solution, but not significantly anisotropic, even in highly birefringent regions of the cell.

REFERENCES

Cotter, M. A. 1977. Hard spherocylinders in an anisotropic mean field: a simple model for a nematic liquid crystal. *J. Chem. Phys.* 66:1098–1106.

Einstein, A. 1956. Investigations on the theory of the Brownian movement. Dover Publications, New York. 122 pp.

Gros, G. 1978. Concentration dependence of the self-diffusion of human and lumbricus terrestris hemoglobin. *Biophys. J.* 22:453–469.

Hanna, S., W. Hess, and R. Klein. 1982. Self-diffusion of spherical Brownian particles with hard-core interaction. *Physica.* 111A:181–199.

Herzfeld, J., A. E. Berger, and J. W. Wingate. 1984. A highly convergent algorithm for computing the orientation distribution functions of rodlike particles. *Macromolecules.* 17:1718–1723.

Hou, L., F. Lanni, and K. Luby-Phelps. 1990. Tracer diffusion in F-actin and Ficoll mixtures: toward a model for cytoplasm. *Biophys. J.* 58:31–43.

Jamieson, A. M., J. G. Southwick, and J. Blackwell. 1982. Dynamical behavior of xanthan polysaccharide in solution. *J. Polym. Sci. Polym. Phys. Rev.* 20:1513–1524.

Marquesee, J. A., and J. M. Deutch. 1980. Concentration dependence of the self-diffusion coefficient. *J. Chem. Phys.* 73:5396–5397.

Muramatsu, N., and A. P. Minton. 1988. Tracer diffusion of globular proteins in concentrated protein solutions. *Proc. Natl. Acad. Sci. USA.* 85:2984–2988.

Reiss, H., H. L. Frisch, and J. L. Lebowitz. 1959. Statistical mechanics of rigid spheres. *J. Chem. Phys.* 31:369–380.

Tough, R. J. A. 1982. Self diffusion in a suspension of interacting Brownian particles. *Mol. Phys.* 46:465–474.

Tracy, M. A., and R. Pecora. 1992. Synthesis, characterization, and dynamics of a rod/sphere composite liquid. *Macromolecules.* 25:337–349.

Wattenbarger, M. R., V. A. Bloomfield, Z. Bu, and P. S. Russo. 1992. Tracer diffusion of proteins in DNA solutions. *Macromolecules.* 25:5263–5265.

Zimmerman, S. B., and A. P. Minton. 1993. Macromolecular crowding: biochemical, biophysical, and physiological consequences. *Annu. Rev. Biophys. Biomol. Struct.* 22:27–66.