CONCENTRATION DEPENDENCE OF PROTEIN DIFFUSION

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ABSTRACT The concentration dependence of protein self-diffusion constants is described by a free volume diffusion theory which accounts for the effects of local protein concentration fluctuations.

INTRODUCTION

Diffusion of proteins plays an important role in such diverse biological processes as enzymatic catalysis and molecular transport. Since functional biological systems may be >30% protein by weight, interpretation of data from dilute solution measurements requires a theoretical understanding of the concentration dependence of the diffusion rate. Most theoretical treatments (1–5) of the hydrodynamics of concentrated protein solutions have been directed toward mutual diffusion. These theories are both mathematically complex and difficult to test as a result of conflicting experimental measurements (6, 7). Two theoretical observations suggest that a simpler approach might be applicable to the study of self-diffusion in concentrated protein solutions. The first is that the dependence of self-diffusion on particle concentration may in principle be described using an equilibrium thermodynamic treatment of the many body interactions (8). The second is that the use of "stick" boundary conditions implies that interacting proteins have a zero velocity of approach at contact (9), which in turn implies that their kinetic energy is dissipated only by protein–solvent interactions, rather than collisions between the protein molecules themselves. Under these conditions, one may apply a theory based on the free volume diffusion model (10–13).

THEORY

Within macroscopically homogeneous solutions there exist microscopic regions in which no protein molecules are found. Assume that within these regions molecules may diffuse freely, as if at infinite dilution. Protein molecules may diffuse only into regions in which sufficiently large vacancies are present. In principle, such a vacancy might be only a fraction of the volume of a protein molecule. In order to avoid performing complex hydrodynamic calculations, however, we assume that these vacancies must be large enough to accommodate both the protein and its hydration shell. Thus, they should be at least as large as the hydrodynamic volume of the protein. If the probability of vacancy formation does not depend on the velocity or direction in which a protein molecule is moving, then the observed diffusion rate will be the product of the dilute solution diffusion rate D_0, and the probability P that there is a void adjacent to the protein which is sufficiently large to permit diffusion

$$D_{obs} = D_0 P. \tag{1}$$

The probability $P(V)$ of forming a vacancy of volume V in the solution is given by the Cohen-Turnbull expression: (10)

$$P(V) = \exp\left[-\gamma\rho V/(1 - \rho V_E)\right], \tag{2}$$

where V_E is a protein exclusion volume (decaliters per gram), ρ is the protein solution density (grams per decaliter), and γ is a constant that determines the ease of vacancy formation. This constant reflects the effects of intermolecular interactions and molecular shape on the probability of vacancy formation, and thus depends on the size and shape of the protein, nature of the solvent, and perhaps other factors.

The probability of forming a cavity of any volume $\int_0^\infty P(V)\,dV$ is normalized to 1, since a cavity of at least size zero must always be present at the protein boundary. Hence,

$$D_{obs} = D_0 \int_{V_E}^{\infty} P(V)\,dV = D_0 \exp\left[-\gamma\rho V_E/(1 - \rho V_E)\right]. \tag{3}$$

This expression reflects only the effects of excluded volume interactions on the diffusion rate and ignores other possible hydrodynamic interactions.

Eq. 3 has the correct behavior at both the low and high protein concentration limits. At low concentrations $\ln(D/D_0)$ decreases linearly with increasing protein concentration, and at sufficiently high protein concentrations D approaches zero, as observed experimentally (14).

There are at least two possible approaches to estimating the protein exclusion volume V_E. The first is to use the hydrodynamic volume V_H (i.e., set $V_E = V_H$), which may be determined from the viscosity using the Einstein relationship (15). This has the advantages of utilizing an independently determinable quantity, as well as of using the same volume in the calculation of the concentration dependence of diffusion as in the calculation of the dilute solution diffusion constant. The second approach, suggested by a referee, is to use an exclusion volume chosen so that the diffusion constant D approaches zero in the limit that ρV_E approaches 1. This exclusion volume could be determined by fitting the data to Eq. 3, or

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by scaling the molecular volume by a factor of 1.4 so that D approaches zero when the protein molecules, assumed to be spherical, approach dense random packing. This appears to be as reasonable as the first approach, but is complicated by difficulties in determining the appropriate protein volume. There are several possible choices, including the partial specific volume (0.0075 dl/g for human hemoglobin), the hard sphere thermodynamic volume (0.009 dl/g for human hemoglobin), and the hydrodynamic volume (0.013 dl/g for human hemoglobin) (16). These choices differ numerically by nearly a factor of 2. Experimental evidence on the diffusion of human and *Lumbricus terrestris* hemoglobin militates against the use of the partial specific volume. The partial specific volumes of human and *Lumbricus* hemoglobins are essentially identical, but the diffusion rate of *Lumbricus* hemoglobin approaches zero at a much lower concentration than that of human hemoglobin (14). No reasonable choice of the scaling factors for dense random packing can accommodate this behavior, nor can any reasonable choice yield an exclusion volume as large as the hydrodynamic volume. For human hemoglobin the excluded volume calculated by the second approach, using the hard sphere thermodynamic volume, is essentially the same as the hydrodynamic volume; since I do not have a hard sphere thermodynamic volume for *Lumbricus* hemoglobin I do not know if use of this volume can yield a reasonable limiting concentration for diffusion of *Lumbricus* hemoglobin.

Because of the difficulty of determining *a priori* the proper volume to use for the second approach, I have chosen to illustrate the utility of Eq. 3 using the hydrodynamic volume for the excluded volume. Use of different volumes suggested by the second approach gives fits that are almost as good, albeit with a different γ. Unfortunately, the ability to fit the data using significantly different excluded volumes makes it difficult to determine the proper molecular volume from the relative goodness of fit.

RESULTS AND DISCUSSION

I fit Eq. 3 to experimental data on the diffusion rate for human hemoglobin (molecular weight ~6,500) published by Gros (14) and by Keller et. al. (7), and to data on the diffusion rate for *Lumbricus terrestris* (earthworm) hemoglobin (molecular weight ~3,700,000) published by Gros. We used for the hydrodynamic volume of human hemoglobin the dilute solution value, 0.013 dl/g (16), and for the hydrodynamic volume of *Lumbricus* hemoglobin the value 0.022 dL/g, obtained from the Einstein relationship and Gros' (14) published viscosity data. For the Keller et al. data, D_0 was set to the largest value they measured experimentally, 8.1×10^{-7} cm^2/s. For Gros' data, D_0 was assumed to be 6.9×10^{-7} cm^2/s (15), and for his *Lumbricus terrestris* hemoglobin data the experimental value for D_0, 1.3×10^{-7} cm^2/s, was used. γ was varied to give the best least squares fit between each data set and Eq. 3.

Figs. 1 and 2 show Gros' (14) experimental results together with the theoretical fits for the concentration dependence of human and *Lumbricus terrestris* hemoglobin diffusion constants, respectively. Agreement between theory and experiment is almost as good for the Keller et al. (7) human hemoglobin data, and the best values for γ (3.0 for the Gros data, 2.8 for the Keller et al. data) are similar. Variation of γ within the range 2.8 to 3.0 does not seriously degrade the quality of the fit to either data set. The excellent agreement between Eq. 3 and the experimental data strongly suggests that the concentration dependence of the protein diffusion constant is governed by excluded volume interactions, which may be predicted by

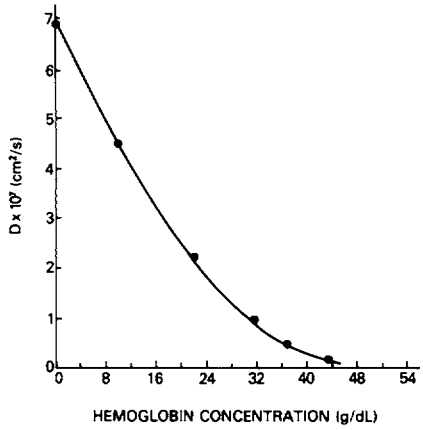

FIGURE 1 Experimental self diffusion rate constants for human hemoglobin (from reference 14), with theoretical curve from Eq. 3 with γ = 3.0.

calculating equilibrium protein density fluctuations. The significant size difference between human and *Lumbricus terrestris* hemoglobin molecules is evidence that this description of the concentration dependence of protein diffusion rates has general applicability.

Eq. 3 is very similar to the Mooney equation (17, 18), which describes the viscosity of concentrated protein solutions when cast in the form

$$\eta/\eta_0 = \exp\{\rho[\eta]/(1 - \rho[\eta](k/v)\}, \quad (4)$$

where η is the viscosity of the solution, η_0 is the solution viscosity at infinite dilution, $[\eta]$ is the intrinsic viscosity of the solution, ρ is the protein solution density, and k/v is a semiempirical constant that corrects for the overlap of free volume.

If one were to equate $[\eta]$ in Eq. 4 with γV_E in Eq. 3, and $[\eta]k/v$ in Eq. 4 with V_E in Eq. 3, then the effects of increasing protein concentration on self-diffusion would be interpreted simply as an effect of increased solution viscosi-

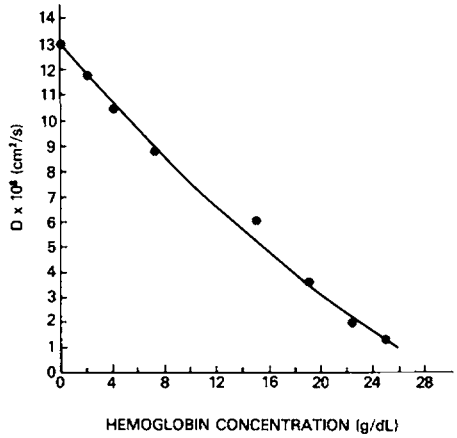

FIGURE 2 Experimental self-diffusion rate constants for *Lumbricus terrestris* hemoglobin (from reference 14), with theoretical curve from Eq. 3 with γ = 1.8.

ty, as observed experimentally (14). While this may be true for single-protein solutions, there is no obvious direct way to derive Eq. 4 from Eq. 3 without making additional assumptions about the measurement of protein viscosity. I believe (see below) that to assume that the concentration dependence of protein diffusion is governed solely by the change in viscosity may be incorrect.

Assume that one measures the diffusion rate of infinitely dilute protein A within a concentrated solution of protein B. From the physical considerations outlined above, the concentration dependence of the diffusion of A at the $\rho_A \rightarrow 0$ limit is given by

$$D^{A}_{obs} = D^{A}_{0} \exp\left[-\gamma_B \rho_B V^{A}_{E}/(1 - \rho_B V^{B}_{E}\right] \quad (5)$$

and D^{A}_{obs} will change at a different rate than D^{B}_{obs}, even though the solution viscosity is governed only by changes in the concentration of B. This forces the conclusion that the exponential terms in Eqs. 3 and 4 may not in general be directly equated.

The product γV_H is very similar for human and *Lumbricus terrestris* hemoglobins, raising the possibility that this quantity may be similar for many proteins. If this constant can be determined independently of the diffusion measurement, it may be possible to predict the concentration dependence of diffusion *a priori*. There is no theoretical reason why this quantity should be conserved for all proteins, since there is a well-known molecular shape dependence of various hydrodynamic properties (15); nevertheless, for similar proteins under similar conditions (for example, hemoglobin and albumin at electrical neutrality) it seems reasonable that this quantity should be similar.

In summary, I have demonstrated that a free volume diffusion theory may be used to accurately and easily describe the concentration dependence of protein self-diffusion rates for proteins having a wide range of molecular dimensions.

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