# The role of diffusion in the binding of carbon monoxide to protoheme in high-viscosity solvents

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Studies of the flash photolysis of heme-bearing proteins have often assessed the role of ligand diffusion in very approximate ways and a disagreement about the importance of ligand diffusion exists in the literature as a result. This paper provides a somewhat more sytematic analysis of diffusional effects than has been given previously for the simple case of ligand–protoheme binding. The model developed here is fit to the available data for the ligand CO in glycerol–water solvents. The fit suggests that diffusional motions become important for the kinetics of this system for temperatures below 270 K and that these motions are strongly correlated when the reactants are close through interactions with the solvent.

## I. INTRODUCTION

The kinetics of rebinding of small ligands to the active sites in heme-bearing proteins subsequent to photodissociation have been actively studied in recent years for the information they contain concerning the form and dynamics of protein structure.[1-14] Unfortunately, some aspects of the interpretation of these rebinding data remain model dependent and several points of interpretative disagreement can be found in the literature. One of the central issues in dispute involves the role that diffusion plays in determining the nature of the rebinding kinetics.[5,7,9,14] Prior discussion of this question seems to have been somewhat casual; typically, one finds conclusions drawn on the basis of comparisons with rate constants[5,7,9] and time courses[9,14] whose applicability for the molecular systems actually under scrutiny demands more careful examination than has been given. The purpose of this paper is to provide a first step toward a systematic analysis of the diffusional effects, which might be associated with the experiments of interest. The formalism presented here is restricted to the analysis of the simplest heme molecule which has been studied, namely, ferroprotoporphyrin IX (protoheme). Because protoheme is free of surrounding protein, its flash photolysis should reveal most directly the effects of ligand diffusion. Treatment of the more complex kinetics associated with heme *proteins* will require, accordingly, more complicated theoretical machinery.

In the next section, the formal details of a model which explicitly incorporates the diffusive motions of protoheme and ligand after photodissociation is presented. Since the experiments of interest deal with small molecules often immersed in solvents of high viscosity, the model emphasizes the effects of solvent "dressing" around the reactant molecules. This is accomplished, in part, by defining more detailed boundary conditions than are normally employed in a typical Smoluchowski equation treatment of diffusion-limited reactions.[15] In Sec. III, the practical utility of the model is tested by applying it to the analysis of experimental data. The most extensive data available in the literature of the type for which the model is appropriate are for the flash photolysis of carbon monoxide bound protoheme in glycerol–water solutions. These data are not sufficiently detailed to require a full implementation of the model; rather, simplifying assumptions can be invoked which yield a reduced model capable of fitting the data with three free parameters. Implications of the parametric values resulting from such a fit and directions for further investigation are discussed in the concluding section.

## II. A KINETICS MODEL FOR LIGAND-PROTOHEME BINDING

The sample of interest is assumed to contain, in solution, protoheme monomers, each of which is initially bound to a ligand of a single type, plus a large excess concentration of ligands of the same type. At $t = 0$, the first photons of a high-intensity flash impinge on the sample breaking ligand-heme bonds. The flash lasts for a time $\tau$, during which very close-lying geminate pairs appear, recombine, and diffusively separate. At the termination of the flash, any surviving geminate pairs which remain spatially close will contribute to an immediate recombination kinetics which will be nonbimolecular and independent of the excess ligand concentration; this kinetic regime ceases when the fraction of such close-lying pairs relative to all pairs in the sample becomes insignificant. Thereafter, the binding kinetics become nongeminate, that is, it will be bimolecular and dependent on the excess ligand concentration. As long as the average initial distance between an *excess* ligand and a protoheme is very much larger than the average initial separation of geminate pair members, which is certainly the case in any real experiment, the geminate phase will be concluded well before this much more gradual, random ligand-heme binding occurs. Consequently, one can formulate the kinetics of these two regimes separately, with an ultimate linkage being accomplished by the requirement that the initial conditions for the nongeminate phase be determined by the infinite time conditions of the geminate phase.

### A. Conditional probabilities

The character of geminate kinetics differs from that of nongeminate kinetics because of the different spatial correlations which prevail between ligand–protoheme pairs during the two regimes. A quantitative discussion

of the different kinetics can be formulated in terms of conditional probabilities for finding a typical unbound ligand in the vicinity of a typical unbound protoheme. Thus, distinct probability densities $\rho_g$ and $\rho_n$ can be defined such that

$\rho_{g[n]}(\mathbf{r}, t) =$ the probability density of finding the geminate [nongeminate] ligand at $\mathbf{r}$ relative to the protoheme reaction center, at time $t$, *given* the protoheme is unbound at $t$.

Since no long-range forces exist between ligand and protoheme, each of the $\rho$'s can be shown[16] to satisfy a generalized Smoluchowski equation, in the bulk solvent, of the type

$$\nabla \cdot (D\nabla\rho_\alpha) + (\partial\rho_\alpha/\partial t)_c = \partial\rho_\alpha/\partial t \ , \quad \alpha = g \text{ or } n \ , \quad (1)$$

where $D$ is the diffusivity of the ligand *relative* to the protoheme and $(\partial\rho_\alpha/\partial t)_c$ represents the probable rate of loss of the ligand due to competitive encounters with all other protohemes. Because both the ligand and the protoheme strongly interact with solvent molecules, which, in turn, interact collectively with the bulk solvent, the motions of the reactant molecules will be correlated when they are close and $D$ will depend, in general, on the relative position of the pair members.[17-24] The competition term for geminate ligands is vanishingly small; for nongeminate ligands it is also small but has an important consequence which will be discussed below. Its form is[16]

$$(\partial\rho_n/\partial t)_c = (\dot{\bar{\rho}}_L/\bar{\rho}_L)\rho_n \ , \quad (2)$$

where $\bar{\rho}_L$ is the sample-average probability density of finding *any* free ligand in the sample at time $t$. Note that the average concentration of free ligands $f_L$ is the maximum possible number of free ligands $N_L$ times $\bar{\rho}_L$.

## B. Boundary and initial conditions

The probability densities $\rho_g$ and $\rho_n$ obey different sets of boundary conditions. For large intrapair separations, $\rho_g$ can be expected to vanish, while $\rho_n$ will approach the sample-average value $\bar{\rho}_L$; i.e.,

$$\rho_g(|\mathbf{r}| \to \infty) = 0 \quad (3a)$$

and

$$\rho_n(|\mathbf{r}| \to \infty) = \bar{\rho}_L \ . \quad (3b)$$

For close encounters (small $|\mathbf{r}|$), the situation is a little more complicated and some care must be exercised in writing down the relevant conditions. The solvent molecules about the disk-like protoheme define a complex, fluctuating "shell of solvation." When the ligand is sufficiently close to the protoheme, this shell encompasses both molecules with some average volume $v$; within this cage the coupling of the ligand and protoheme to the surrounding fluid is reduced. In order to balance mathematical tractability with physical reality, assume that the solvation shell can be replaced equivalently by a sphere of equal volume centered on the heme iron and that spherical symmetry prevails about that center. The dynamics of the ligand, protoheme pair for separations less than $R$, the radius of the solvation sphere, is extremely complicated, of course. For the

purposes of this paper it is sufficient to assume that $v\rho_\alpha(R, t)$ is the probability of finding the tagged ligand within $R$, given the protoheme is unbound, and that when the ligand is within $R$ of the (postulated) unbound protoheme its probable rate of binding is first order and can be expressed as $kv\rho_\alpha(R, t)$, where $k$ is a phenomenological, first-order rate constant. The probable rate of transfer of the ligand to or from the solvent surrounding the (postulated) unbound protoheme is assumed to be governed by diffusive flux which will be negative (outward flux) for geminate and positive (inward flux) for nongeminate ligands. Because binding is assumed to occur only on the "front" side of the protoheme, only about half of the surface defined by $v$ is available for these diffusive fluxes. The appropriate transfer rate is then approximately $2\pi R^2 D(R)\partial_r\rho_\alpha(R, t)$. Finally, during the duration of the flash pulse free ligands and unbound protohemes are created with separations less than $R$. If $\phi(t)$ denotes the probable rate of creation of such geminate pairs, where $\phi > 0$ for $0 < t < \tau$ and $\phi = 0$ for $t > \tau$, then

$$v\partial_t\rho_g(R, t) = \phi(t) - kv\rho_g(R, t) + 2\pi R^2 D(R)\partial_r\rho_g(R, t) \quad (4a)$$

and

$$v\partial_t\rho_n(R, t) = -kv\rho_n(R, t) + 2\pi R^2 D(R)\partial_r\rho_n(R, t) \quad (4b)$$

are the desired boundary conditions on $\rho_g$ and $\rho_n$ at $r = R$.

Initially, there are no geminate pairs and the assumption of a uniform distribution of ligands and protohemes is sufficient to describe the initial stages of nongeminate binding; therefore,

$$\rho_g(r \geq R, t = 0) = 0 \quad (5a)$$

and

$$\rho_n(r \geq R, t = 0) = \bar{\rho}_L \quad (5b)$$

are the appropriate initial conditions for which Eq. (1) must be solved.

## C. Connection with observed kinetics

The raw binding rate data in a typical photolysis experiment can be cast in terms of the probable rate at which binding occurs. Let $p_\alpha(t)$ designate the probability that, on the average, a protoheme is bound at $t$ (in the appropriate regime) and $q_\alpha(t)$ $(= 1 - p_\alpha)$ that it is unbound. The measured rates are then $\dot{p}_\alpha$ or $\dot{q}_\alpha$; these are connected to the conditional probabilities $\rho_\alpha$ as follows.

In general, the probable rate of binding between a protoheme and a ligand is given by the product $kP(R)$, where $P(R)$ is the probability that *any* unbound protoheme and *any* free ligand are within $R$ of each other. In the geminate regime, the probability of finding an unbound ligand within $R$ of any protoheme (bound or not) is dominated by contributions due to geminate pair partners. And, since competition with other ligands for the protoheme is ignorable, the survival of the ligand assures that the protoheme also remains unbound during this regime. Hence, $P(R)$ is identical to $v\rho_g(R, t)$ for geminate binding.

For nongeminate binding, the spatial correlation

which formerly existed between an unbound protoheme and its geminate pair ligand has been erased by diffusive escape. Consequently, each of the (approximately) $N_L$ ligands contributes equally to the calculation of $P(R)$ at late times. Furthermore, because of competition among the ligands for each protoheme there is no guarantee that when a ligand and a protoheme do pass within $R$ of each other that the protoheme remains unbound (given that it was initially). Thus, to compute $P(R)$ one needs to form the product: (Probability of *any* ligand being within $R$ of a protoheme given the protoheme is unbound)×(probability the protoheme actually is unbound.) In other words, $P(R) = [N_L v \rho_n(R, t)] q_n(t)$, where the factor $N_L$ results from summing over contributions from all unbound ligands.

These considerations lead finally to expressions for the observed rates in terms of the conditional probabilities defined previously:

$$\dot{p}_g = -\dot{q}_g = -\phi(t) + kv\rho_g(R, t) \tag{6a}$$

and

$$\dot{p}_n = -\dot{q}_n = [kN_L v\rho_n(R, t)] q_n(t) . \tag{6b}$$

These kinetic relations are supplemented with the initial conditions

$$p_g(t = 0) = 1 \tag{7a}$$

and

$$p_n(t = 0) = p_g(t = \infty) . \tag{7b}$$

Equation (7b) provides the means by which the two regimes are smoothed together.

## III. APPLICATION OF THE MODEL

To demonstrate its utility, the model described above can be applied to the high temperature ($T \geq 230$ K) data of Alberding et al.[5] (called "A et al." subsequently) for the flash photolysis of CO from protoheme in glycerol-water. The data look qualitatively like that depicted in Fig. 1. In the experiment reported by A et al., data begin to be observed 2 $\mu$s after the flash, which has a 1 $\mu$s duration, is initiated. Details of rapid geminate kinetics are not seen. The flat plateaus at early times correspond to the fractions of protohemes surviving geminate recombination at different temperatures. These values correspond to the $q_g(t = \infty)$ of the previous section. For notational simplicity, these escape fractions shall hereafter be denoted by $\epsilon$. The downward sweeping curves at late times can be fit by simple exponentials of the form $q_n = \epsilon \exp(-\lambda t)$, where the rate constants $\lambda$ are observed to be proportional to the excess CO concentration.[5] This behavior bears the signature of a pseudo first-order nongeminate binding process. Since the data permit one to extract the temperature dependence of both $\epsilon$ and $\lambda$, fitting these quantities with sensible parameters represents a major test of the model outlined in Sec. II.

Determination of the forms of $\epsilon$ and $\lambda$ associated with the proposed model, requires the integration of Eqs. (6a) and (6b), which in turn necessitates solution of the diffusion Eq. (1). Successful completion of this task
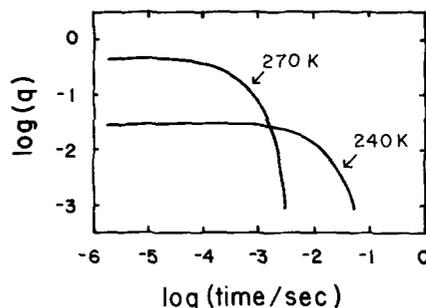


FIG. 1. Schematic representation of typical kinetics data from Ref. 5. The fraction of protohemes still unbound is plotted as a function of time. The curves correspond to nongeminate ligand binding. The early geminate binding processes are completed before the first data are recorded.

depends on knowledge of the forms of both the diffusivity $D(r)$ and the production function $\phi(t)$. In its most general form, this problem represents a formidable exercise.

Fortunately, though undoubtedly at the cost of some accuracy, the complexity of the problem at hand can be reduced substantially by invoking the following assumptions.

### A. Simplifying assumptions

(i) In the experiment of interest, the excess CO concentration is sufficiently high that one can treat the sample-average concentration of free CO molecules as essentially constant. Under this condition, the nongeminate probability will rapidly achieve a steady state and $\partial_t \rho_n(r, t)$ can be assumed to vanish for all $r$.

(ii) The hydrodynamic interaction between two diffusing molecules is predicted, theoretically, to reduce the rate of their collisional encounter and escape.[17-20,22] It has been shown[19,20] that for large Brownian particles which have achieved a stationary state, this hydrodynamic repulsion effect can be adequately described by replacing the relative diffusivity $D(r)$ in Eq. (1) by a spatially invariant effective diffusivity $D'$. Such a replacement is assumed to be valid here as well, even though the molecules of interest are hardly Brownian particles and the geminate phase is clearly not a steady state.

(iii) A et al., in analyzing their data, employ a model in which $\phi(t)$ has the form of a delta function $\delta(t - \tau)$. Such a form cannot be justified for the model proposed here. Typical diffusive relaxations occur in times on the order of $R^2/D'$. If $R$ is a few angstroms and $D'$ takes on typical values between, say, $10^{-9}$ and $10^{-5}$ cm$^2$ s$^{-1}$, $R^2/D'$ will be less than the flash pulse width of 1 $\mu$s reported in A et al. Thus, nontrivial dynamics occur during the flash and its finite duration must be taken into account. To this end, $\phi$ is assumed to have the simple phenomenological form

$$\phi(t) = \begin{cases} \sigma p_g(t) , & 0 < t < \tau \\ 0 , & t > \tau , \end{cases} \tag{8}$$

where $\sigma$ is some effective rate constant. In general,

TABLE I. Temperature dependences of the viscosity of glycerol–water (3 : 1, $v/v$), the first-order binding rate constant for CO–protoheme, and the effective diffusive relaxation time.

| $T(K)$ | $\log(\eta/cp)^a$ | $k(10^8 \ s^{-1})$ | $R^2/D'$ ($\mu$s) |
|---|---|---|---|
| 340 | 0.87 | 4.88 | $1.26 \times 10^{-3}$ |
| 330 | 1.00 | 4.69 | $1.77 \times 10^{-3}$ |
| 320 | 1.15 | 4.49 | $2.59 \times 10^{-3}$ |
| 310 | 1.32 | 4.30 | $3.98 \times 10^{-3}$ |
| 300 | 1.55 | 4.10 | $6.96 \times 10^{-3}$ |
| 290 | 1.82 | 3.91 | $1.33 \times 10^{-2}$ |
| 280 | 2.10 | 3.71 | $2.61 \times 10^{-2}$ |
| 270 | 2.44 | 3.52 | $5.93 \times 10^{-2}$ |
| 260 | 2.82 | 3.33 | $1.48 \times 10^{-1}$ |
| 250 | 3.25 | 3.14 | $4.23 \times 10^{-1}$ |
| 240 | 3.78 | 2.95 | 1.46 |
| 230 | 4.28 | 2.77 | 4.88 |

[a]See Ref. 8.

$\sigma = \sum_{i,f} \sigma_{if} p_i$, where $\sigma_{if}$ is the probable rate of transition to a free state $f$ from a bound state $i$, due to photon absorption and $p_i$ is the canonical ensemble probability that the initially bound complex is in state $i$. The intrinsic rate $\sigma_{if}$ depends, among other things, on photon energy and polarization, protoheme orientation, and the instantaneous intensity of the pulse, and would be difficult to evaluate from first principles. On the other hand, the quality of the geminate data to be analyzed does not demand such an evaluation and $\sigma$ will here be taken to be a temperature-independent, time-averaged, free parameter.

(iv) With the aid of assumptions (i)–(iii), Eqs. (6a) and (6b) can be integrated by numerical techniques, though the task is still nontrivial. One further simplification which leads to closed form, analytic solutions for $\epsilon$ and $\lambda$ can be invoked, however. This stems from the recognition that a solution to Eq. (1) with boundary condition (3a) and initial condition (5a) evolves so that $\partial_r \rho_g(R, t)$ rapidly approaches $-\rho_g(R, t)/R$, for times greater than $R^2/D'$ (see the Appendix). For values of $R^2/D'$ much less than the length of the pulse (1 $\mu$s), the gradient term in Eq. (4a) can be replaced by a term proportional to just $\rho_g(R, t)$ to excellent approximation. For $R^2/D'$ values on the order of 1 $\mu$s (see Table I) this approximation is probably not so excellent but is made here for simplicity anyway.

## B. Numerical results

Assumptions (i)–(iv) yield a great reduction in the complexity of the coupled Eqs. (1), (4), and (6). The reduced forms of the observed kinetics relations [Eq. (6)] are

$$\dot{p}_g = -\dot{q}_g = \begin{cases} -\sigma p_g + kv\rho_g(R, t) , & 0 < t < \tau \\ kv\rho_g(R, t) , & t > \tau \end{cases} \quad (6'a)$$

and

$$\dot{q}_n = -\lambda q_n , \qquad (6'b)$$

where the constant $\lambda$ is $kN_L v\rho_n(R)$, $\rho_n$ being assumed

stationary. Clearly, Eq. (6'b) leads to qualitative agreement with the kinetic behavior depicted in Fig. 1. The reduced forms of the boundary conditions (4) are

$$v\partial_t \rho_g(R, t) = \begin{cases} \sigma p_g - k_T v\rho_g(R, t) , & 0 < t < \tau \\ -k_T v\rho_g(R, t) , & t > \tau \end{cases} \quad (4'a)$$

and

$$0 = -kv\rho_n(R) + 2\pi R^2 D' \partial_r \rho_n(R) . \qquad (4'b)$$

In Eq. (4'a) the quantity $k_T$ is

$$k_T = k + k_D , \qquad (9)$$

while

$$k_D = 2\pi RD'/v . \qquad (10)$$

[Equation (4'b) can be recognized as the so-called "radiation boundary condition"[25]; its validity can be seen to be restricted to the vanishing of $\partial_t \rho_n(R, t)$.]

Solving Eq. (1) for $\rho_n(r)$ with the conditions $\rho_n(\infty) = \bar{\rho}_{CO}$ and Eq. (4'b) leads to

$$\lambda = 2\pi RD' F f_{CO} , \qquad (11)$$

where

$$F = (1 + k_D/k)^{-1} \qquad (12)$$

and $f_{CO} = N_{CO} \bar{\rho}_{CO}$ is the sample-average, excess CO concentration. Equations (4'a) and (6'a) are a coupled set which can be solved in time steps $0 < t < \tau$ and $\tau < t$. The result for $\epsilon = q_g(\infty)$ is

$$\epsilon = 1 - A \exp(-a\tau) - B \exp(-b\tau) , \qquad (13)$$

where

$$\begin{pmatrix} a \\ b \end{pmatrix} = \{(k_T + \sigma) (\pm) [(k_T + \sigma)^2 - 4k_D\sigma]^{1/2}\}/2 , \qquad (14)$$

$$A = (a - k_T - F\sigma)/(a - b) , \qquad (15)$$

and

$$B = (k_T + F\sigma - b)/(a - b) . \qquad (16)$$

In addition to $\sigma$, how many free parameters are available in Eqs. (11) and (13) to fit the data? The candidates would seem to include $R$, $D'$, $v$, and $k$ ($f_{CO}$ is given in A et al.). However, the low temperature ($T < 80$ K), data of A et al. can be taken as an independent determination of $k$. For the purposes of this paper, $k$ will be assumed to be of the transition state theory form,[26] $k = (k_B T/h) \exp(\Delta S/k_B) \exp(-\theta/T)$, where, of course, $k_B$ and $h$ are the Boltzmann and Planck constants, respectively, and $\Delta S$ and $\theta$ are the activation entropy and temperature, respectively, for CO–protoheme binding. In terms of the quantities defined in A et al., $\Delta S = S_{aa}^{peak}$ and $\theta = H_{aa}^{peak}/k_B$. Furthermore, if $R_0$ is defined as the sum of the covalent radii of an Fe atom and a CO molecule, then $v$ and $R$ are related through $v = 4\pi(R^3 - R_0^3)$. Thus, there are only three parameters which are unknown, namely, $R$, $D'$, and $\sigma$.

While $R$ and $\sigma$ may be assumed to be essentially temperature independent, $D'$ will obviously vary (perhaps, rapidly) with $T$. Caldin and Hasinoff[9] have claimed that in their experiments, the nongeminate constant $\lambda$ varies

as $T/\eta$ over some temperature range and indeed the slope given by A *et al.* of $T/\eta$ and $\lambda$ (called $\lambda_{IV}$ there) do look very similar below 270 K. Since $D' \propto \lambda$ as $F \to 1$, according to Eq. (11), it may be reasonable to assume that $D' = D_0 T/\eta$, where $D_0$ is an unknown constant. Implications of such a temperature dependence will be discussed in Sec. IV.

The viscosities of glycerol–water mixtures of varying composition and temperature can be found in the extensive data of Beece *et al.*[8] Values relevant to a (3 : 1, v/v) solution are found in Table I. The values of $\Delta S$ and $\theta$ for protoheme–CO in such a solvent are, from A *et al.*, $\Delta S/k_B = -9.21 \pm 1.15$ and $\theta = 126 \pm 25$ K; the CO concentration in this experiment is $f_{CO} = 2.4 \times 10^{17}$ cm$^{-3}$. The radius $R_0$ is taken to be 2.5 Å.

The rate constant $\lambda$ can be fit as a function of temperature to the data using the values quoted above in a non-linear least squares routine. Best fit values of $D_0$ and $R$ are produced by the procedure. Similarly, a best fit value of $\sigma$ results from fitting the escape fraction $\epsilon$ as a function of temperature using the $D_0$ and $R$ from the $\lambda$ fit. The latter procedure is facilitated by recognizing that $k > 10^8$ s$^{-1}$ over the temperature range of interest (see Table I); thus, $k_T \tau \gg 1$ for all $T$ and, hence, Eq. (13) becomes simply $\epsilon = 1 - B \exp(-b\tau)$. The fits of $\lambda$ and $\epsilon$ are shown in Figs. 2 and 3. The best fit numerical values for the free parameters are

$$\sigma = 5.6 \times 10^6 \text{ s}^{-1} ,$$

$$R = 4.1 \pm 1.9 \text{ Å} ,$$

$$D_0 = 2.8 \times 10^{-8} \pm 0.8 \times 10^{-8} \text{ cm}^2 \text{cp s}^{-1} \text{K}^{-1} .$$

The standard deviations in these values *associated with the fitting routine* are found to be extremely small. The reported experimental uncertainties in $\Delta S$ and $\theta$, however, lead to substantial uncertainties in all three parameters. The uncertainties cited for $R$ and $D_0$ originate in the experimental uncertainty. The associated uncertainty in $\sigma$ is at least a factor of three.

## IV. DISCUSSION

The model presented in this paper attempts to provide a theoretical context for judging the role of diffusion in the binding of ligands to protoheme. A number of ap-
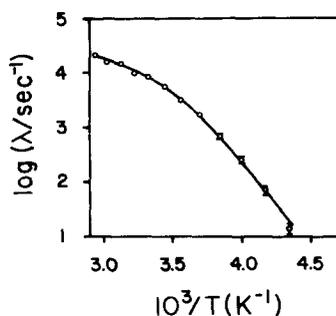


FIG. 2. The nongeminate binding rate constant is plotted as a function of reciprocal temperature. The circles are data points taken from Ref. 5. The solid curve is a fit of the data using Eq. (11) of the text.
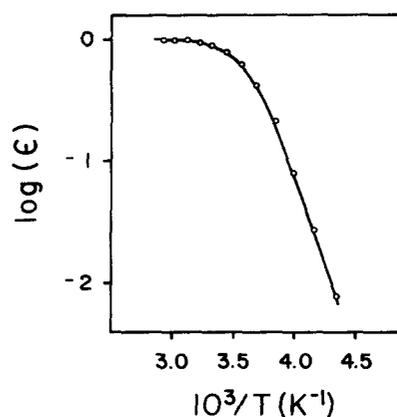


FIG. 3. The fraction of geminate pairs escaping immediate recombination is plotted as a function of reciprocal temperature. The circles are data points taken from Ref. 5. The solid curve is a fit of the data using Eq. (13) of the text.

proximations have been invoked to produce a tractable analysis. The merits of these need appraisal before any conclusions can be formulated.

First, there is the question of geometry. Ligands have been pictured here as diffusing into and out of a spherical volume surrounding the heme iron, through effectively half of the bounding surface. This simplification of the actual geometry can be expected to overestimate the diffusive in and outflows and result in a fitted value of $D_0$, which is too low. As long as the bounding surface is relatively open, however, this error is probably not great.[27]

Estimates of $\sigma$ are affected by two assumptions. The replacement of $\partial_r \rho_g(R, t)$ by $-\rho_g(R, t)/R$ in Eq. (4a) is strictly valid only if $\tau \gg R^2/D'$. Examination of the values of $R^2/D'$ tabulated in Table I reveal that this condition is only really well-satisfied for $T \ge 270$ K. The replacement below 270 K *underestimates* the diffusive outflow of geminate ligands. On the other hand, the hydrodynamic interaction between protoheme and ligand is likely to result in $D(R)$ being less than $D'$. Hence, the assumption $D(r) \to D'$ probably produces an *overestimation* of the diffusive outflow and, hence, a compensation for the former approximation.

Since the simplifications used in the analysis of Sec. III appear to be reasonable the parametric values of $\sigma$, $R$, and $D_0$ should have at least order of magnitude accuracy. The plausibility of the best fit values for $\sigma$ and $R$ is easily established. Indeed, the value for $R$ requires no comment. Alberding *et al.*[5] have fit their data with a somewhat different model from the one proposed here, assuming that the flash pulse had the shape of a delta function. Such a pulse time averaged over a 1 $\mu$s interval leads to an effective $\sigma$ value of $10^6$ s$^{-1}$, in rough agreement with the value quoted in Sec. III.

Interpretation of the parameter $D_0$ is not as straightforward. The relative diffusivity $D(r)$ should approach the sum of the independent diffusivities of protoheme and CO as $r \to \infty$. Mounting experimental evidence seems to indicate that the diffusion of small molecules in high-viscosity solvents may be faster than that pre-

dicted by the Stokes–Einstein relation,[28–32] in which $D \propto T/\eta$; in fact, it has recently been argued that $D \propto T/\eta^p$, where the power $p$ is less than 1 and depends on the solute.[31,32] Departures from Stokes–Einstein behavior have also been hinted at in several theoretical works.[33–35] Why, then, the $D'$ of Sec. III should yield such a close fit to the glycerol–water data requires further investigation. One possible explanation might be that the departure of the diffusivity of a small molecule from Stokes–Einstein behavior is due to microscopic, short wavelength, nonhydrodynamic processes,[33–35] whereas the final diffusive step of a ligand toward a protoheme requires a collective "shearing" of solvent molecules across the flat protoheme disk, a process which may be much more hydrodynamic like. If the latter process is rate limiting at low temperatures, then the effective diffusivity $D'$ might well be dominated by hydrodynamic $T/\eta$ behavior. (This is, of course, conjectural and needs to be demonstrated.) In light of these arguments, a proportionality of the rate constant $\lambda$ with $T/\eta$, therefore, may *not* be a signature of diffusion control (in the usual sense), as is sometimes alleged, but, rather, may be an artifact of "cage structure rearrangement."

To complete this discussion of the parameter $D_0$, a crude estimate of its magnitude may be obtained by modeling the *close approach* of CO and protoheme molecules as follows. Since close approach may be hydrodynamically limited, let both molecules be visualized as Brownian spheres of hydrodynamic radii $r'_{CO}$ and $r'_H$ interacting through a continuous fluid. Under these conditions $D_0$ would have the form

$$D_0 = \nu k_B (1/r'_{CO} + 1/r'_H) , \qquad (17)$$

where $\nu$ is a dimensionless number, the value of which depends on the boundary conditions between the fluid and the "molecular" spheres. For "slip" conditions, $\nu \doteq (0.7) \times (1/4\pi)$; for "stick" conditions $\nu \doteq (0.5) \times (1/6\pi)$. In each case, the first factor is due to hydrodynamic repulsion.[19,20] Taking, say, $r'_{CO} = 2$ Å and $r'_H = 5$ Å yields a range of possible $D_0$ values:

$$2.6 \times 10^{-8} \text{ cm}^2 \text{ cp s}^{-1} \text{ K}^{-1} < D_0 < 5.4 \times 10^{-8} \text{ cm}^2 \text{ cp s}^{-1} \text{ K}^{-1} ;$$

these are certainly compatible with the best fit value of $2.8 \times 10^{-8}$ cm$^2$ cp s$^{-1}$ K$^{-1}$ cited previously.

To summarize, a theoretical model for the kinetics of rebinding of small molecules in a liquid solvent subsequent to flash photolysis has been outlined in Sec. II. Aided by what has been argued are plausible simplifying assumptions, the model has been fit to the CO–protoheme (in glycerol–water) data of Alberding et al.,[5] utilizing three free parameters. The best fit values of these parameters have been shown to be reasonable. The temperature dependence of the nongeminate binding rate constant has been shown to be very nearly given by $T/\eta$ below 270 K. Assuming that CO diffusivity in glycerol–water *does* depend on temperature differently from $T/\eta$ (this has to be demonstrated), the rate limiting step below 270 K has been provisionally identified as associated with short-range correlations of the reactant motions due to caging effects.[36] A much better test of the model presented here, vis-a-vis other models,

awaits (i) better theoretical understanding of the spatial and temperature dependence of $D(r)$, (ii) faster time resolution of geminate kinetics, and (iii) precise experimental determination of the viscosity dependence of CO diffusion.

## ACKNOWLEDGMENTS

## APPENDIX

During the flash, the geminate probability density $\rho_g$ satisfies

$$D' \nabla^2 \rho_g = \partial_t \rho_g , \qquad (A1)$$

subject to the conditions

$$\rho_g(r = \infty, t) = 0 \qquad (A2)$$

and

$$\rho_g(r, 0) = 0 . \qquad (A3)$$

The Laplace transform of Eq. (A1) may be solved easily to obtain

$$\hat{\rho}_g(r, s) = A(s) \exp(-qr)/r , \qquad (A4)$$

where $\hat{\rho}_g$ is the Laplace transform of $\rho_g$, $s$ is the transform variable, and $q^2 = s/D'$. Clearly, Eq. (A4) implies

$$\partial_r \hat{\rho}_g(R, s) = -(qR + 1)\hat{\rho}_g(R, s)/R . \qquad (A5)$$

For the time regime corresponding to $qR \ll 1$ (for real $s$), Eq. (A5) can be inverted to yield

$$\partial_r \rho_g(R, t) = -\rho_g(R, t)/R , \qquad (A6)$$

the result used in Sec. III. The relation $qR \ll 1$ is equivalent to $R/(sD')^{1/2} \ll 1/s$, which upon inversion leads to $(D't)^{1/2} \gg R$. The latter expression defines the time domain for which Eq. (A6) is valid.

[1]The reviews by L. J. Parkhurst in Ann. Rev. Phys. Chem. **30**, 503 (1979) and by F. R. N. Gurd and T. M. Rothgeb in Adv. Protein Chem. **33**, 73 (1979) contain many pertinent references.

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[36]This conclusion agrees qualitatively, at least, with the models of Refs. 5 and 8 which also emphasize the importance of caging effects.