Hypothesis

Structural interpretation of low-temperature heme-ligand recombination rates in myoglobin

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The nonexponential recombination of photodissociated heme-CO and heme-O_2 in myoglobin, which is geminate at $T < 180$ K, is interpreted as being due to a narrow, random distribution of ligand transfer distances in the heme pocket. This permits evaluation of the most probable recombination rate which is shown to be consistent with ligand tunneling.

Myoglobin *Heme-ligand recombination* *Ligand tunneling* *Protein disorder* *Conformational substate*

1. INTRODUCTION

The ability of heme-proteins to bind and release O_2 and CO is not only of vital biological importance, but also provides a means to monitor protein dynamics. This was exploited by Frauenfelder et al. [1–4] and others [5,6] who studied the time dependence of heme-O_2 and heme-CO recombination following photodissociation. At low temperatures ($T < 180$ K), the ligands recombine with the Fe atoms to which they were originally attached, implying that they do not leave the heme pocket. However, this 'geminate' recombination is nonexponential; multiple-flash experiments have shown that this is due to the inhomogeneity of the heme-protein system [1]. The presence of many conformational substates, each with its own characteristic recombination rate constant k, leads naturally to a distribution of rate constants $F(k)$. The observed nonexponential time dependence is the Laplace transform of this distribution:

$$f(t) = k_0^{-1} \int_0^\infty F(k) \exp(-kt) dk \qquad (1)$$

There have been several attempts to relate the observed $f(t)$, or the distribution $F(k)$ derived from it by inversion of eqn 1, to the structure and dynamics of the protein substates [1,5,6]. Typically, one tries to relate $F(k)$ to a distribution of heights of barriers obstructing the recombination. On the basis of plausible physical arguments, Agmon and Hopfield [5] proposed a Gaussian distribution which, however, does not provide a good fit to the data. Young and Bowne [6] achieved a much better fit with an asymmetrical (gamma) distribution; however, this distribution involves an additional parameter and lacks a clear physical foundation.

2. MODEL

In this paper, we propose a new interpretation which differs from previous efforts in that it is based on structural rather than dynamic properties of the system. It has the added advantage of not being restricted to the process at hand but applying to a wide variety of relaxation processes in disordered systems [7,8]. One of these, the so-called hole burning in absorption spectra of dyes dissolved in glasses [9,10], closely resembles heme-ligand photodissociation: in both instances, absorption of a photon creates a localized disturbance which leaves the system in a metastable state; its subsequent relaxation occurs at a rate that

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is site-dependent. Such processes can be interpreted formally in terms of a two-level system [11,12] in which the initial and final levels are separated by a barrier; the relaxation is described as 'tunneling' through this barrier. Adopting this description, we express the tunneling rate in terms of the tunneling distance, i.e., the separation of the initial and final equilibrium positions of the tunneling atoms. If this separation is large compared to the vibrational amplitudes of these atoms, the tunneling rate will be governed by the wings of the vibrational wave functions, in accordance with the Franck-Condon principle. Since these wings and thus their overlaps fall off exponentially, this yields [7,13]

$$k = k_0 \exp[-\lambda(R - R_0)], \quad (2)$$

where R is the tunneling distance and λ a parameter depending on the mass of the tunneling atoms. An isotopic mass dependence has indeed been observed for heme-CO recombination at low temperatures [14].

To represent the inhomogeneity of the system, we introduce a distribution $\Phi(R)$ of tunneling distances. To derive the form of this distribution, we make the following 'natural' assumptions: (i) in a perfectly ordered environment $R = R_0$ so that $\Phi(R)$ reduces to $\delta(R - R_0)$; (ii) the deviations from

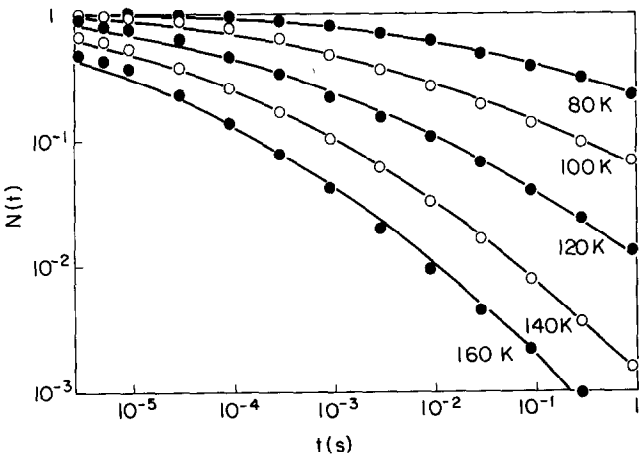

Fig.1. Recombination kinetics of myoglobin-CO in 75% glycerol-water at pH 7. The data points show the relative concentration of unreacted CO taken directly from fig.4 of [6]. The solid lines provide the best fits to the data obtained with eqn 5 for the parameter values listed in table 1.

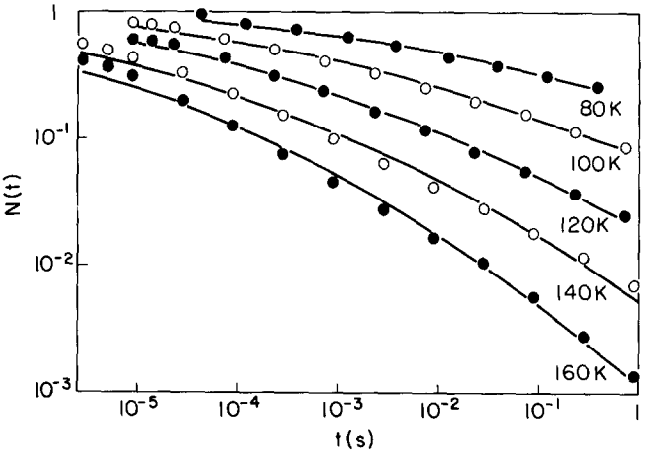

Fig.2. Same as fig.1 for myoglobin-O_2 with data points taken directly from fig.6 of [6].

R_0, arising from the presence of conformational substates in the experimental environment, are random and small compared to R_0; and (iii) these random deviations are independently distributed, i.e., the substates are quasi-independent. Then $\Phi(R)$ may be approximated by a Gaussian distribution

$$\Phi(R) = \pi^{-1/2}\, \Gamma^{-1} \exp[-(R - R_0)^2/\Gamma^2] \quad (3)$$

with width Γ. Substitution of eqn 2 yields

$$F(k) = \pi^{-1/2}\, \Gamma^{-1} \exp\{-[\ln(k/k_0)/\lambda\Gamma]^2\} \quad (4)$$

with the Laplace transform

$$f(t) = \pi^{-1/2}\, \gamma^{-1} \exp(-\tfrac{1}{4}\gamma^2) \int_{-\infty}^{\infty} \exp(x - x^2/\gamma^2 - k_0 t e^x)\mathrm{d}x, \quad (5)$$

where $x = \ln(k/k_0) = -\lambda(R - R_0)$ and $\gamma = \lambda\Gamma$.

As shown elsewhere [7,8], eqn 5 yields an excellent fit to many relaxation processes in disordered systems, including spectroscopic hole burning. We therefore compare it with kinetic heme-ligand recombination data obtained by Frauenfelder et al. [1,6] for myoglobin. The results are displayed in fig.1 for heme-CO and in fig.2 for heme-O_2. The data points represent the observed concentrations $N(t)$ of the unattached ligand (i.e., 'free' CO or O_2) at time t relative to $N(0) \equiv 1$. They were taken directly from figs 4 and 6 of [6], respectively. The curves in figs 1 and 2 were calculated

from eqn 5; physically meaningful values of k_0 and γ were obtained by minimization of the sum of squared relative deviations of $f(t)$ from the data points, including the point $N(0) = 1$. No plot is presented for data points at $T = 60$ K, since their range of $N(t)$ values is insufficient to fix k_0 and γ reliably.

3. DISCUSSION

Given the simplicity of the model, we consider the fits displayed in figs 1 and 2 to be very good. The model slightly overestimates the fraction of ligands recombining with the heme during the first few microseconds. Although the fit can be improved by modification of eqn 2 and/or 3, this generally requires additional adjustable parameters. This increase in flexibility is not justified for the available data sets.

If the structural parameters in eqns 2 and 3 are assumed to be temperature-independent, the temperature dependence of k_0 and γ, displayed in fig.3, should be governed by $\lambda(T)$. If we further assume that eqn 2 may be generalized to $k_0(R_0) \simeq k_0(0)\exp(-\lambda R_0)$ with $k_0(0)$ temperature-independent, we have $\mathrm{d} \ln k_0/\mathrm{d}\gamma = -R_0/\Gamma$. The data in fig.3 imply that $R_0/\Gamma \simeq 15$ for both heme-CO and heme-O_2, consistent with our basic assumption that the spread in tunneling distances is small compared to the average distance. Also, the linear dependence of $\ln k_0$ on T rather than $1/T$ is typical for tunneling transfer [13,15]. We note that $\mathrm{d} \ln k_0/\mathrm{d}T$ for heme-O_2 exceeds $\mathrm{d} \ln k_0/\mathrm{d}T$ for heme-CO by a factor 1.12 which is close to the ratios $(M_O/M_C)^{1/2} = 1.15$ and $(M_{O_2}/M_{CO})^{1/2} = 1.07$, where M represents the atomic or molecular mass. This is consistent with the isotopic mass effect for myoglobin-CO reported by Alben et al. [14], but not with the model proposed by Jortner and Ulstrup [16] in which the Fe atom of the heme is the tunneling particle.

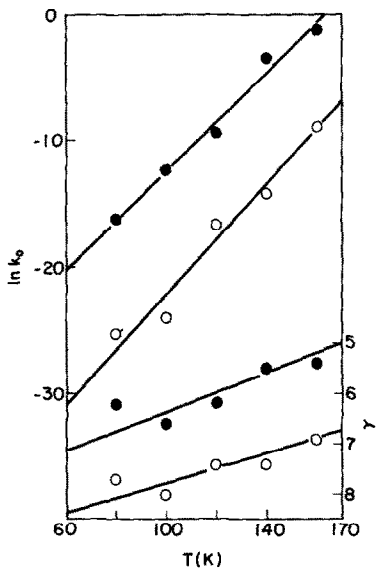

Fig.3. Temperature dependence of the most probable rate constants k_0 and the effective widths γ of the distributions $F(k)$, given by eqn 4, for myoglobin-CO (●) and myoglobin-O_2 (○).

Since our model leads to the definition of the most probable rate constant k_0, and especially its temperature dependence, it provides direct information on the nature of the recombination process. For obvious reasons the barrier that obstructs recombination will be multi-dimensional. As a result, there will be many different paths through and over the barrier, such that in general the faster the rate the smaller will be the Boltzmann factor since barriers tend to narrow towards the top. As shown elsewhere [7,13], summation over these pathways typically leads to a quasi-linear dependence of $\ln k$ on T in the appropriate temperature domain. This behavior, displayed in fig.3, has also been observed for many hydrogen tunneling reactions [13]. On the other hand, simple one-dimensional barrier models, which generally predict an Arrhenius-type temperature dependence, fail to reproduce the kinetic data of figs 1 and 2. This was recognized by Frauenfelder et al. [3] who pointed out that the classical Kramers treatment [17], in which the dependence of the rate of transfer over a barrier on coordinates other than the reaction coordinate is introduced via a damping term, is much more relevant. Based on the same philosophy but expressed in quantum-mechanical language, our treatment relates the slope $\mathrm{d} \ln k_0/\mathrm{d}T$ in fig.3 to the vibrational potentials whose intersection generates the multi-dimensional barrier. This relationship between the temperature dependence of the recombination rate and the shape of the heme pocket will be explored quantitatively in a forthcoming paper.

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