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On the mechanism of ligand binding to myoglobin

The role of structural fluctuations

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Abstract. The association reaction of CO and O_2 with heme is expected to reflect the differences in the electronic structures of the two ligands. CO binding should be controlled by a high spin/low spin transition while oxygen binding is spin-allowed. Dioxygen should thus bind substantially faster than CO. The experimental association rates of the two ligands are, however, almost identical. We propose that the reaction is triggered in both cases by a fast structural intermediate which allows the CO molecule to bind adiabatically. A suitable structural transition has been identified recently by inelastic neutron scattering.

Key words: Heme proteins, nonadiabatic reactions, protein dynamics, neutron scattering

I. Introduction

The association reaction of small ligands such as O_2 and CO with myoglobin is a superb probe to investigate static and dynamic effects of the protein matrix on the heme group. Ligand binding results in a transition of the heme from a domed to a planar structure and a corresponding displacement of the iron by 0.45 Å (Takano 1977; Phillips 1980). Besides numerous nonbonded contacts the heme is linked covalently to His-F8, suggesting a tight coupling between matrix motions and displacements of the heme group. The association reaction has been studied over a wide range of temperatures by flash photolysis experiments (Austin et al. 1975) resulting in the following reaction scheme (Doster et al. 1982):

$$A \leftarrow B \rightleftharpoons S + L\,.$$

A is the initial and final state, when the ligand L is bound to the heme iron. A laser flash breaks the bond, the free state in the heme pocket is denoted by B. From B the ligand can bind directly or move into the solvent $S+L$ via intermediate states which are not discussed here. The on-rate λ_{on} denoting the recombination with ligands initially in the solvent $(S+L)$ is given by (Doster et al. 1982):

$$\lambda_{\text{on}} = k_{BA}\, N^{\text{out}} P_B\,. \qquad (1)$$

N^{out} is the fraction of ligands reaching the solvent following a laser flash and P_B, the pocket occupation factor, denotes the equilibrium coefficient of $B \rightleftharpoons S+L$ in the absence of $B \rightarrow A$. λ_{on} is proportional to the rate of the final binding step k_{BA}. The exchange of ligands with the solvent is frozen below 200 K and only the process $B \rightarrow A$ is observed. The survival fraction of free ligand in B has a power law time dependence in contrast to exponential kinetics expected for an elementary unimolecular reaction. This observation is taken as evidence that $B \rightarrow A$ is modified by a multiplicity of conformational states interconverting slowly at low temperatures (Austin et al. 1975; Young and Bowne 1984; Agmon and Hopfield 1983; Bialek and Goldstein 1985; Ansari et al. 1985; Champion 1988).

Our concern is the microscopic mechanism of this reaction which is still controversial. It is not understood why the two ligands O_2 and CO, despite different electronic structures, bind with almost the same average speed (Frauenfelder and Wolynes 1985). The free CO molecule is a closed shell ligand while O_2 has unpaired electrons outside a close shell. As carbon monoxide approaches the iron it destabilizes the high spin deoxy state of the heme and lowers the energy of the singlet state. The quintet-singlet transition is spin forbidden. Binding resulting in a singlet state must occur via a triplet intermediate which could be mediated by spin-orbit coupling (Jortner and Ulstrup 1979; Redi et al. 1981). In this case one expects the reaction to be non-adiabatic. Oxygen binding by contrast is spin allowed since the iron in oxymyoglobin has spin 1 which is paired with the spin of O_2. This leads to an overall singlet state. Electronic changes should follow the nuclear rearrangements adiabatically. A criterion to estimate the degree of adiabacity of a reaction was given by Landau and Zehner (1932)

$$\gamma_{LZ} = \frac{\Delta}{\hbar/\tau_{LZ}} . \tag{2}$$

The adiabaticity parameter γ_{LZ} denotes the ratio of the energy splitting Δ between the two diabatic energy curves (quintet-singlet) to the energy uncertainty of the system which is given by the lifetime τ_{LZ} in the quintet-singlet mixing region. The reaction is adiabatic for large splitting $\gamma_{LZ} \gg 1$ and non-adiabatic if $\gamma_{LZ} \lessapprox 1$. The probability P for the electronic structure to change on single crossing is then given by:

$$P = 1 - \exp(-\pi \gamma_{LZ}/2) . \tag{3}$$

Frauenfelder and Wolynes (1985) obtain: $\gamma_{LZ}(O_2) = 6$ and $\gamma_{LZ}(CO) = 10^{-2}$.

According to Eq. (3) CO should bind at least 60 times slower than O_2 in contrast to the experimental result. The authors suggest the existence of a hitherto unobserved intermediate state which could explain an adiabatic formation of the CO complex.

The problem of non-adiabatic CO binding was analyzed by Jortner and Ulstrup (1979) using a Born-Oppenheimer approximation. Their essential assumption was that the spin-orbit coupling $\Delta \approx 1-10 \text{ cm}^{-1}$ is small compared to the frequency of vibrations which induce the transition in the nuclear coordinates. They assume $\omega_{\text{vib}} \approx 200 \text{ cm}^{-1}$ which is about the wave number of the iron-His stretching mode (Nagai et al. 1980). Thus $\tau_{LZ} \approx \omega_{\text{vib}}^{-1}$ is small.

As discussed below neutron scattering and Mössbauer spectroscopy reveal the existence of non-vibrational motions with longer lifetimes. The effect of such fluctuations on ligand binding is the subject of this work.

II. The model

We assume that the association reaction is triggered by a structural transition whose life time τ_{LZ} is long enough to guarantee the adiabatic limit. We expect this fluctuation to distort the domed deoxy-structure of the heme towards a planar geometry. An immediate consequence of this assumption is that both ligands should bind at approximately the same speed within a factor of two, CO binds with the carbon and O_2 can bind both ways, and with small energetic differences due to interactions of the ligand in the heme pocket.

Consider the following kinetic mechanism:

$$B_1 \underset{k_{01}}{\overset{k_{10}}{\rightleftharpoons}} B_0 \xrightarrow{k_{0A}} A . \tag{4}$$

B_1 is the ground state and B_0 denotes the distorted deoxy-structure. k_{0A} measures the availability of the ligand for the reaction during the lifetime of B_0. The association rate is then given by:

$$k_{BA} = \frac{k_{10}}{k_{01} + k_{0A}} k_{0A} . \tag{5}$$

To proceed further requires an identification of the structural transition which triggers the reaction.

The vast number of possibilities can be restricted by the following properties:

1) A dynamic coupling to the heme group

2) A lifetime which is just about consistent with the adiabaticity criterion since the unstable, distorted deoxy-structure cannot have a long lifetime.

3) Consistency with the temperature dependence of k_{BA} according to Eq. (5)

In the following we examine a particular set of motions in myoglobin which were identified recently by inelastic neutron scattering (Doster et al. 1989). Neutron scattering probes the motion of protons which are uniformly distributed throughout the protein. The resulting spectrum is complex and covers several decades in frequency. We focus on the high frequency component termed the β-process which is the fastest non-vibrational process. β can be modelled by the motion of a particle in an asymmetric two-state potential (Fig. 1) (Doster et al. 1989). The low energy state may reflect a minimum in energy corresponding to the X-ray structure. The energy asymmetry explains the observed increase in motional amplitude with temperature. The proton displacements are substantial (1.5 Å), probably rotational jumps in the dihedral angles. The correlation time of this process ranges between 0.5 and 1 ps almost independent of the temperature. To examine whether this process affects the heme we plot (Fig. 2) its mean squared displacement together with $\langle \Delta x^2 \rangle_{\text{Fe}}$ of the iron determined by γ-resonance absorption (Parak et al. 1982; Knapp et al. 1982). Both data sets were fit using the asymmetric two-state model. The resulting energy asymmetries are

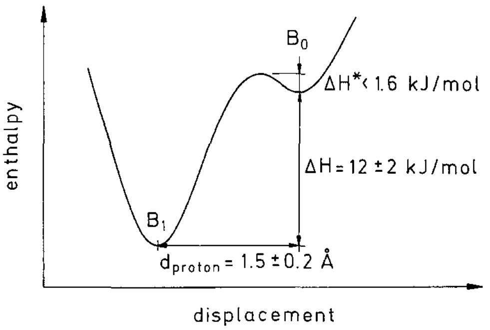

Fig. 1. Asymmetric two-state model of the fast structural transition used to analyze the mean squared displacements of the protons and the heme iron and the kinetics of ligand binding according to Eq. (5)

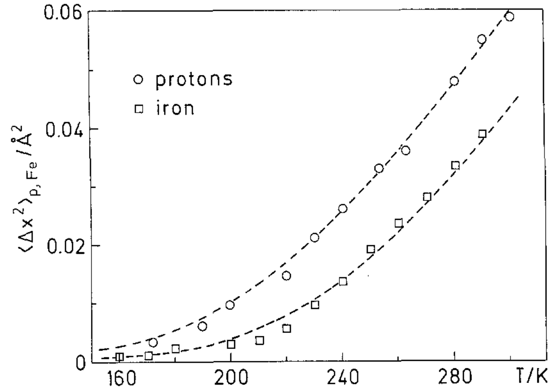

Fig. 2. Mean squared displacements of protons in H_2O hydrated myoglobin powder (0.34 g/g) (Doster et al. 1989) and of the iron in myoglobin crystals (about the same hydration) (Knapp et al. 1982). The vibrational contribution was subtracted in both cases. The *dotted lines* correspond to fits to the two state model of Fig. 1

similar: 12 ± 2 kJ/mol (protons) and 13.7 kJ/mol (iron). It is reasonable that the iron has a smaller mean squared displacement. The differences in energy and the deviations in the fit are probably related to the different time resolution of the two methods, 10^{-7} s (γ) versus 10^{-10} s (n). The parallel temperature dependence of the two data sets is consistent with the β-process contributing to the displacement of the iron. A major contribution of a fast process to $\langle \Delta x^2 \rangle_{Fe}$ has been proposed by Bauminger et al. (1983).

We now examine the adiabaticity criterion. The crucial point is the lifetime τ_{LZ} in the AB mixing region. τ_{LZ} is determined by the lifetime of B_0. This quantity amounts to 0.5 ps if we identify the two states of the β-process with B_0 and B_1. Using the parameters given by Redi et al. (1981) $\Delta(\mathrm{CO}) = 10\ \mathrm{cm}^{-1}$ and $\Delta(O_2) = 200\ \mathrm{cm}^{-1}$ we obtain: $\gamma_{LZ}(O_2) = 20$ and $\gamma_{LZ}(\mathrm{CO}) = 1$. According to Eq. (3) this amounts to a 20% difference in the speed of recombination between CO and O_2 triggered by the fastest component of the non-vibrational spectrum. $\tau_{LZ} = 1$ ps would give a 5% difference. The correlation time of the β-process is therefore sufficient to explain almost identical binding rates.

The next point is the temperature dependence of k_{BA} derived from Eq. (5). As mentioned above it is likely that the contribution of the ligand to the reaction is mainly entropic. This implies a nearly temperature independent rate constant k_{0A}. Since the linewidth of the β-process does not vary with temperature it follows that k_{01} is also nearly temperature independent. Thus in both limiting cases $k_{01} \gg k_{0A}$ and $k_{01} \ll k_{0A}$ we expect the energy asymmetry of about 12 kJ/mol to dominate the temperature dependence of k_{BA}. This number has to be compared to the peak activation enthalpy of process $B \rightarrow A$ obtained from flash photolysis experiments: O_2: 10.5 kJ/mol, CO: 11 kJ/mol (Frauenfelder and Wolynes 1985). At this stage one can conclude that the β-process is a suitable candidate in a scenario which assumes that ligand binding is induced by a non-vibrational structural transition.

III. Discussion and conclusion

We have given, in the case of sperm whale myoglobin, a fairly detailed picture of a possible intermediate state which according to Frauenfelder and Wolynes (1985) may solve the puzzle of ligand-independent binding rates. We first discuss what happens when we extend the data base. At first sight, if the β-process is a characteristic feature of protein motions, the model predicts protein-independent ligand binding kinetics. Comparing myoglobins from different species it seems that 10–11 kJ/mol is a characteristic number. Even "mini-myoglobin" prepared from horse heart myoglobin by proteolytic treatment (about one third is removed) is consistent with this statement (Di Iorio 1988). However, the four human hemoglobin chains all have peak activation energies of about 4 kJ/mol. This result suggests the involvement of another type of structural transition. Microwave absorption experiments on hemoglobin reveal at least three processes on a subpicosecond timescale which are analyzed by similar asymmetric two-state potentials (Genzel et al. 1983). The corresponding energy asymmetrics are 1) 1 kJ/mol, 2) 3.7 kJ/mol, and 3) 13 kJ/mol. These processes contribute to the microwave absorption between 300 and 50 K. Process 3 has common features with the β-relaxation. Process 2 could explain the binding in hemoglobin chains.

We are pointing out that the energy differences controlling the motional amplitudes of the picosecond dynamics seem to correlate with the apparent activation energies in the binding kinetics. This implies that the in- and out of plane motion of the heme iron should occur on a picosecond time scale. Femtosecond absorption spectroscopy of photodissociated hemoproteins have shown a high spin intermediate which relaxes with a time constant of 350 fs. This process is tentatively attributed to the out of plane motion of the heme iron (Martin et al. 1983). With this result one can expect that the fluctuations involved in the reverse process occur on the same time scale.

So far we have ignored the non-exponential features of process $B \rightarrow A$. The possible microscopic origin of the activation energy distribution has been discussed by the authors mentioned in the introduction. We would like to add a new aspect to this discussion, since it is generally assumed that freezing of slow structural motions near 200 K is directly linked to the evolution of an activation energy spectrum. This view ignores that in cases where process $B \rightarrow A$ can be observed above 200 K it still has non-exponential kinetics (Ansari et al. 1986). Since, according to our model, the reactive fluctuations are fast, the protein structure on that time scale is effectively frozen at all temperatures. At high temperatures however, the energy spread in the energy asymmetry caused by slow motions is on the order of RT resulting in exponential kinetics. The situation changes gradually with decreasing temperature. It follows for instance that solvent motions should have no effect on k_{BA}, explaining why $B \rightarrow A$ in myoglobin is independent of the solvent viscosity (Beece et al. 1980). The continuity of process $B \rightarrow A$ with temperature is also expressed by the apparent activation energy of λ_{on} (Eq. (1)) which is about 12 kJ/mol (Doster et al. 1982). $\lambda_{\mathrm{on}}^{-1}$ at 300 K and atmospheric CO pressure in 75% glycerol/water is 1.4 ms. Analyzing the data using Eqs. (1) and (5) one obtains the following numbers:

$$P_B \approx 10^{-4}, \quad N^{\mathrm{out}} = 1, \quad k_{10}/k_{01} \approx 10^{-2}$$
$$\text{and} \quad k_{0A} \approx 10^9\ \mathrm{s}^{-1}.$$

In this way one can understand how a millisecond event relates to sub-picosecond protein motions. The relatively small prefactor $k_{0A} = 10^9\ \mathrm{s}^{-1}$ (compared to $10^{13}\ \mathrm{s}^{-1}$) may be a consequence of the reaction mechanism which requires a coincidence of a $B_1 \rightarrow B_0$ transition and a correct position and orientation of the ligand in the heme pocket. Further insight into this question may come from molecular dynamic simulations of structural motion in the vicinity of the heme group. Finally, it should be mentioned that the dissociation rate λ_{off} critically depends on the differences in the electronic structures of the two ligands. However, the adiabatic binding mechanism proposed here contributes to the biological potency of CO as a metabolic poison, by enhancing its association rate constant, λ_{on}.

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