Pressure Effects on Electron Transfer Rates in Zinc/Ruthenium Modified Myoglobins

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The pressure dependence of the intramolecular electron transfer rates in myoglobin was measured to examine the control mechanism of the biological electron transfers through protein fluctuations. Over the past few years, interests in the effects of pressure on the electron transfer (ET) rates in proteins have been increased,[1,2] since pressure can perturb various determinants of the ET rate constants involved in the Marcus equation, from which the control mechanism of the ET could be deduced. Two previous studies on pressure dependence of the intramolecular ET rates have been reported in hemoproteins.[1,2] These studies found out that the electron transfer rates observed for cytochromes *c* and b_5 are a little accelerated by pressurization or almost independent of pressure, depending on proteins and electron transfer pathways.[1,2] Pressure-induced increase in the ET rate can be interpreted in terms of an increased electronic coupling term caused by the contraction of the through space distance along the ET pathway.[1] However, the factors which would affect the electron transfer in protein have not been fully understood. Particularly, effects of dynamic motion on electron transfer in protein have never been examined, although the dynamic motion has been suggested to play key roles in the reaction.[3] In the present study, we have investigated the pressure effects on the intramolecular ET rate for myoglobin derivatives, which is in contrast to cytochromes *c* and b_5 in the sense that myoglobin is more susceptible to the perturbation of the dynamic motion than the cytochromes as is manifested by its high compressibility.[4]

We have synthesized some pentaammineruthenium (a_5Ru) modified and zinc porphyrin (ZnP) reconstituted human myoglobins (a_5RuZnPMb)[5−10] to examine pressure dependence of the ET rate from photoexcited ^{3}ZnP* to a_5Ru (see inset of Figure 1).[5] The distance between ZnP and a_5Ru (D−A distance) is varied from 9.5 to 19.3 Å by the following site-directed mutagenesis of myoglobin; H48N/H81N/T70H (Ru-modified at His70), H81N (His48), H48N/H81N/E83H (His83) and H48N (His81). Figure 1 illustrates ^{3}ZnP* decay signals for a_5Ru-(His48)ZnPMb at 0.1 and 100 MPa, both of which could be fitted entirely by a single exponential curve. It is apparent that the ET rate (k_{ET}) for a_5Ru(His48)ZnPMb is smaller at 100 MPa than that at 0.1 MPa. Table 1 summarizes the observed k_{ET} for a_5RuZnPMb derivatives at the atmospheric pressure and their D−A distances estimated from the X-ray structure.[5,6] The k_{ET} decreases exponentially with the D−A distance, and the distance decay factor, β, was estimated as 1.3Å^{-1}.[11] Figure 2 illustrates the pressure dependence of the observed k_{ET} for a_5RuZnPMb derivatives that indicates that the pressurization decelerated k_{ET}

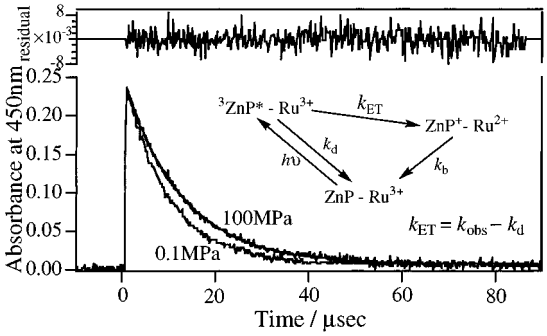

Figure 1. Traces: Transient absorbance changes obtained after the laser excitation (532 nm; 10-ns pulse width) of ZnP in a_5Ru(His48)-ZnPMb at 0.1 and 100 MPa. The experiments were performed at 293 K. The sample solution contained 10 μM of myoglobin derivative dissolved in 100 mM Tris-HCl buffer at pH 7.4. The pH of Tris buffer was shown to be independent of pressure up to 200 MPa.[20] Absorbance changes were monitored at 450 nm, where ^{3}ZnP* contributes mainly. The smooth line is a fit to an single exponential function of the data obtained at 100 MPa. The residual of the fitting is shown on the top. Inset: Schematic representation of the intramolecular electron transfer cycle of a_5RuZnPMb. The electron transfer rate constant, k_{ET}, was obtained as the difference between the observed decay rate constant, k_{obs}, and the intrinsic decay rate constant of ^{3}ZnP*, k_d. The latter was estimated separately using a ZnP incorporated myoglobin without the a_5Ru modification.

Table 1. The D−A Distance, k_{ET}, and $\Delta V^{\ddagger}$ for the Intramolecular Electron Transfer Process in a_5RuMbZnP Derivatives

Ru-modified residue	D−A distance/Å	k_{ET}/s^{-1}	$\Delta V^{\ddagger}$/cm^{3}mol^{-1}
His70	9.5	5.05×10^7	+4
His48	12.7	1.03×10^5	+6
His83	15.5	1.18×10^3	+9
His81	19.3	5.52×10^1	+17

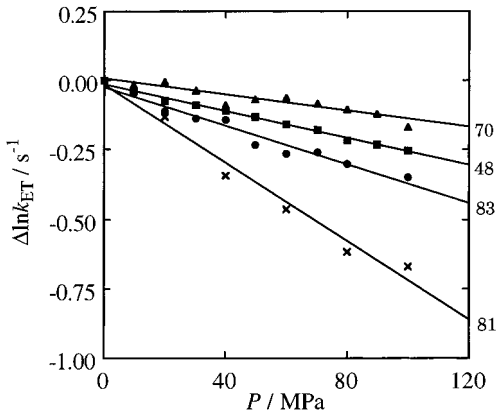

Figure 2. Pressure dependence of intramolecular ET rates in a_5-RuMbZnP derivatives. Numbers in the figure denote the position of the Ru-modified site. The ordinate values (Δ ln k_{ET}) are presented as the changes in ln k_{ET} from that at the atmospheric pressure. Solid lines are linear least-squares fits of the experimental data.

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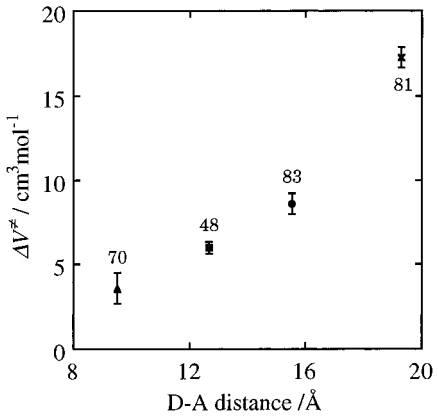

Figure 3. D−A distance dependence of activation volumes. Numbers in the figure denotes the position of the Ru-modified site.

for all the derivatives. The activation volumes, calculated by the relation,

$$\Delta V^{\ddagger} = -RT\left(\frac{\partial \ln k_{ET}}{\partial P}\right)_T$$

increase with increasing the D−A distance as is shown in Table 1 and Figure 3. The positive activation volumes are in sharp contrast to those observed in cytochromes *c* and b_5.[1,2] Meier *et al.* suggested that application of pressure on cytochrome *c* causes slight compression of through space gaps, an increase of the electronic coupling term and eventually the acceleration of the ET rate.[1] In myoglobin, however, the deceleration of the electron transfer implies that the compression of the space gap in the electron pathway is not the only factor which induces the pressure effects on the reaction rate.

As the compressibility experiments[4] suggested, the structure of myoglobin is more flexible than those of cytochromes *c* and b_5. Our previous high-pressure NMR studies on hemoproteins have also revealed that myoglobin is susceptible to the pressure and denatures at the lower pressure than cytochrome *c*.[12] These observations suggest that the protein flexibility, that is, the thermal fluctuation, would be one of the characteristic factors that might explain the pressure dependence of electron transfers in myoglobin. The effect of thermal fluctuation can be introduced into the electronic coupling term, H_{AB}, as follows. If the structure of myoglobin is fluctuating, the actual ET distance (r) would also fluctuate around the D−A distance (d) estimated from the X-ray crystal structure. Let us suppose that its potential energy is $\alpha(r-d)^2$, where α is the curvature coefficient of the potential energy. The electronic coupling term, H_{AB}, which is expressed as a function of d ($H_{AB} = H^{\circ}_{AB}\exp(-\beta d)$) should be averaged over the Boltzmann factor, $\exp[-\alpha(r-d)^2/kT]$. The resulting electronic coupling term is given by the equation 1. In this equation, the factor $\exp(kT\beta^2/4\alpha)$

$$H_{AB} = H^{\circ}_{AB}\exp\left(-\beta d + \frac{kT\beta^2}{4\alpha}\right) \quad (1)$$

comes from the contribution of the thermal average and is referred to as the thermal fluctuation factor.[13]

Under high pressure, the hydrostatic compression increases material density and suppresses the thermal fluctuation and fluctuation width.[14] Decreasing the fluctuation width corresponds to increasing the curvature coefficient α. Accordingly, high pressure is supposed to increase α, which in turn depresses the thermal fluctuation factor $\exp(kT\beta^2/4\alpha)$. It, then, follows that the high pressure may decelerate the ET rate, k_{ET}, through the electronic coupling term. The positive activation volume of ET in myoglobin can therefore be explained qualitatively by including the thermal fluctuation factor in the electronic coupling term H_{AB}. Thermal fluctuations also explain that activation volumes depend on the D−A distance, since α is more substantially affected by pressure at the larger D−A distances that leads to the larger changes in k_{ET}.

We have ignored so far the effect of pressure on ΔG° and λ that are also important determining factors of the electron transfer process.[15,16] While both of the factors can be dependent on pressure,[17] we consider that the effects are secondary compared to that on the electronic coupling term, H_{AB}. The values for $-\Delta G^{\circ}$ and λ of the present system at the atmospheric pressure are rather similar (0.82 and 1.26 eV, respectively[18]) that make k_{ET} relatively insensitive to the variations of ΔG° and λ. In order to explain the decrease of k_{ET} observed for His81 derivative in terms of ΔG°, unrealistic increase of ΔG° as much as 0.09 eV upon pressurization is necessary. Furthermore, this change of ΔG° must be less than 0.02 eV for His70 derivative. To a first approximation, both ΔG° and λ should show the pressure dependency which is little correlated with the donor−acceptor distances, since they are mainly governed by the local circumstances around redox centers.[19]

In summary, the ET rate is decreased with increasing pressure in all the a_5RuZnPMbs. The activation volume is positive and depends on the D−A distance, which is in sharp contrast to cytochromes *c* and b_5. Since myoglobin is more flexible than cytochromes b_5 and *c*, which is considered to cause its unique pressure dependency, we can suggest that the thermal fluctuation term, $\exp(kT\beta^2/4\alpha)$, modulates the electronic coupling term H_{AB} for the electron transfer in myoglobin. The present interpretation is hypothetical and must be tested by further experiments as well as by rigorous theoretical treatment which includes the effect of pressure on ΔG° and λ. We believe, however, that this is the first experimental demonstration of the fluctuation controlled electron transfers observed in biological systems.

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(19) ΔG° depends on the distance between donor and acceptor, which is a result of the different Coulombic interaction between the redox partners.[15] Similarly, the solvent contribution of λ (λ_0) also depends on the D−A distance.[15] However, the distance dependency of the biological electron transfers has been analyzed successfully without incorporating these effects.[11,16,18]

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