II-D Structure and Function of Metalloproteins and Its Molecular Design

Metal ion is a common cofactor that is crucial for active centers of proteins involved in many biologically important processes in cells, and a relatively small number of metal-based prosthetic groups are utilized to serve numerous and diverse chemical functions. A typical metal-based prosthetic group, which represents a fascinating example in this respect, is heme. Heme promotes a variety of functions, such as dioxygen storage, activation of small molecules, electron transfer reactions, and sensing gaseous molecule. In the field of protein design and engineering, hemoproteins also make particularly attractive targets. There are many reasons for this, including the exciting possibility of engineering protein-based molecules with useful catalytic, electronic or optoelectronic properties. Based on various kinds of spectroscopies, we have functionally and structurally characterized some hemoproteins including newly identified heme-regulated proteins, and designed hemoproteins showing improved activities and new functions.

II-D-1 Absence of a Detectable Intermediate in the Compound I Formation of Horseradish Peroxidase at Ambient Temperature

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A microsecond-resolved absorption spectrometer was developed to investigate the elementary steps in hydrogen peroxide (H_2O_2) activation reaction of horseradish peroxidase (HRP) at ambient temperature. The kinetic absorption spectra of HRP upon the mixing with various concentrations of H_2O_2 (0.5–3 mm) were monitored in the time range from 50 to 300 μs. The time-resolved spectra in the Soret region possessed isosbestic points that were close to those between the resting state and compound I. The kinetic changes in the Soret absorbance could be well fitted by a single exponential function. Accordingly, no distinct spectrum of the putative intermediate between the resting state and compound I was identified. These results were consistent with the proposal that the O–O bond activation in heme peroxidases is promoted by the imidazolium form of the distal histidine that exists only transiently. It was estimated that the rate constant for the breakage of the O–O bond in H_2O_2 by HRP is significantly faster than 1×10^4 s^{-1}.

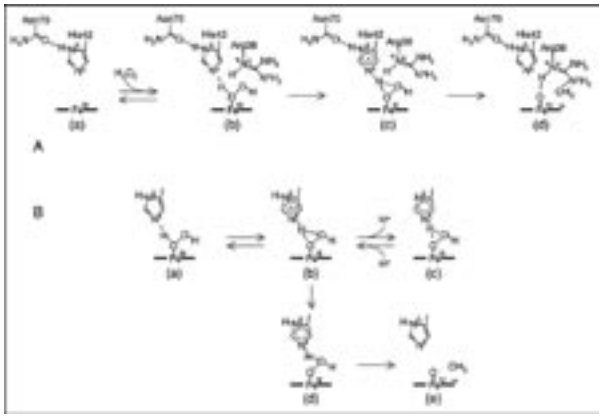

Figure 1. The proposed formation mechanisms of compound I. *A*, the formation mechanism of compound I in heme peroxidases proposed by Poulos and Kraut. The amino acid numbering is for HRP, although the original proposal was based on the structure of cytochrome *c* peroxidase. *(a)*, the resting state. *(b)* and *(c)*, the hypothetical intermediate and transition state structures for the compound I formation. His-42 acts as a general acid-base catalyst and translocates a proton from the proximal to the distal oxygen. *(d)*, compound I. The hydrogen bonds between oxygen atoms and Arg-38 were detected in the recent crystallographic data. *B*, the generalized mechanism of hydrogen peroxide activation by heme proteins described by Egawa *et al. (a)*, the hydrogen peroxide-bound form. *(b)*, the transition state for the O–O bond heterolysis. The structure is unstable because of the doubly protonated His. *(c)*, the deprotonation of the distal His causes the stabilization of the iron hydroperoxide complex. *(d)*, the proton translocation occurs only from the structure (*(b)*). *(e)*, compound I.

II-D-2 Dehydration in the Folding of Reduced Cytochrome *c* Revealed by the Electron-Transfer-Triggered Folding under High Pressure

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We determined the activation volume associated with protein folding of reduced cytochrome *c* from the collapsed intermediate to the native state. The folding rate was followed by a change in the absorption (420 nm) at various pressures between 0.1 and 200 MPa and at various concentrations of denaturant (guanidine hydrochloride) between 3.2 and 4.0 M. Dependence of the folding rate on both these factors revealed that the activation volume at ambient pressure in the absence of denaturant is negative ($\Delta V^{\ddagger} = -14 \pm 7$ $cm^3 \cdot mol^{-1}$). Such a negative activation volume can be accounted for by a decrease in volume resulting from the dehydration of hydrophobic groups, primarily the heme group. Dehydration, which increases the entropy of the protein system, compensates for a decrease in the entropy

accompanying the formation of the more compact and ordered transition state. We, therefore, propose that the positive change in the activation entropy for the folding reaction is due to the dehydration of hydrophobic groups. Furthermore, dehydration entropically promotes the protein folding reaction.

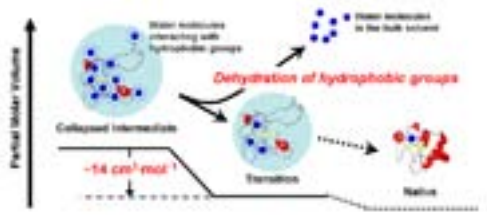

Figure 1. Volume profile for protein folding of cytochrome *c*.

II-D-3 Time-Resolved Small Angle X-Ray Scattering Investigation on the Folding Dynamics of Heme Oxygenase: Implication of the Scaling Relationship for the Submillisecond Intermediates of Protein Folding

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Polypeptide collapse is generally observed as the initial folding dynamics of proteins with more than 100 residues, and is suggested to be caused by the coil-globule transition explained by Flory's theory of polymers. To support the suggestion by establishing a scaling behavior between radius of gyration (R_g) and chain length for the initial folding intermediates, the folding dynamics of heme oxygenase (HO) was characterized by time-resolved, small-angle X-ray scattering. HO is a highly helical protein without disulfide bridges, and is the largest protein (263 residues) characterized by the method. The folding process of HO was found to contain a transient oligomerization; however, the conformation within 10 ms was demonstrated to be monomeric and to possess R_g of 26.1±1.1 Å. Together with the corresponding data for proteins with different chain lengths, the seven R_g values demonstrated the scaling relationship to chain length with a scaling exponent of 0.35±0.11, which is close to the theoretical value of 1/3 predicted for globules in solutions where monomer-monomer interactions are favored over monomer-solvent interactions (poor solvent). The finding indicated that the initial folding dynamics of proteins bears the signature of the coil-globule transition, and offers a clue to explain the folding mechanisms of proteins with different chain lengths.

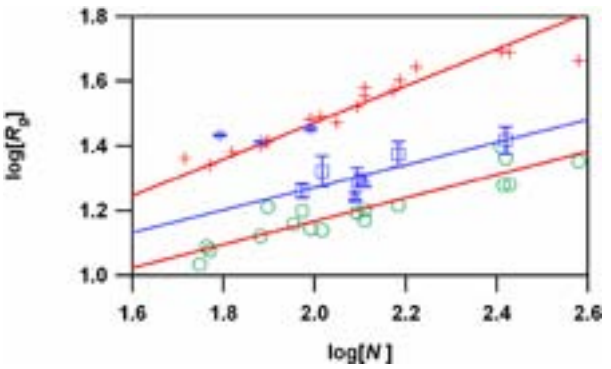

Figure 1. The correlation plots between R_g and chain length (N) for the chemically unfolded states (crosses), native states (circles) and initial collapsed intermediates (squares and triangles) of various proteins. Squares and triangles represent R_g values obtained with the submillisecond and millisecond time resolutions, respectively. Lines denote the scaling relationships for the respective states. The data for the chemically unfolded state, and for the native state, are reported elsewhere.