THERMAL UNFOLDING OF A FERRI-CYTOCHROME C

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Thermal unfolding (structural melting) of ferri (Fe^{3+})-cytochrome c of several animals has been investigated by using of 500MHz ^1H nuclear magnetic resonance (NMR) spectroscopy and high-sensitive differential scanning calorimetry (DSC). We find that the unfolding process of these proteins depends on the animal species (i.e. difference in amino acid sequences) and can be classified into two types. (1)1-step unfolding : thermal destruction of the native structure starts at same low temperature simultaneously and everywhere in a molecule, then proceeds, with increasing temperature, gradually and more and more destructively, and eventually becomes the fully denatured state above the transition temperature. (2) Multi-steps unfolding : local destruction of the native structure takes place successively at several temperatures and places within a molecule with increasing temperature, and then follows destruction of 3-dimensional structure as a final step with the wide transition region($\Delta T \sim 20℃$).

Cytochrome c is a small globular protein with a diameter of 3.4nm (a ultra fine particle) and functions as an electron-transport enzyme at mitchondrial membrane in cells. Cytochrome c consists of a single polypeptides chain of 104 amino acid residues and a covalently attached heme group as shown in Fig.1. There are five α-helical segments and is no β-pleated sheet. Its 3-dimensional structure is heterogeneous and is delicately balanced and stabilized, in the physiological condition (the native state), by noncovalent competing interactions such as hydrogen bonding, Coulomb's force, van der Waals force and hydrophobic effect. When a protein is heated to 90℃, the native structure is destroyed and melts, accompanying with absorption of (latent) heat, into random coil. This phenomenon is explained as a cooperative (1st order) phase transition between the native and denatured states (2 states transition model). According to this picture, only a ratio of these two kind of molecule changes during the phase transition. Because 3-dimensional conformation of a protein is constructed by the arrangement of some building blocks (a segment of polypeptides) with different thermal strength, melting of the native structure seems to occur successively from some thermally weak region in a molecule. Thus it is a

fundamental question to ask how "cooperatively" does the structure destruction proceed within a complex molecule.

^{1}H-NMR spectroscopy is highly appropriate for the study in thermal unfolding and structural change of a protein, because the protein includes many protons which act as a local (NMR) probe and these proton signals are very sensitive to a conformational change. Fig.2 shows the high frequency region of 500MHz ^{1}H-NMR spectra of Bovine heart ferri-cytochrome c as a function of temperature. In the room temperature each signal appears at such chemical shift positions as that reflects the native conformation of the protein. When the protein is heated up to 80℃, the intensity of each signal decreases gradually and eventually disappears above T_c (= 77℃ transition temperature). Decrease and/or disappearence in the signal intensity corresponds to local and/or global (large scale) destruction of the native structure. Remaining broad signals above T_c reflects the conformation not of the native but of the random coil structure.

In the meeting we report also DSC data and discuss about thermal unfolding (structural phase transition) of a small protein from a view point of complex systems.

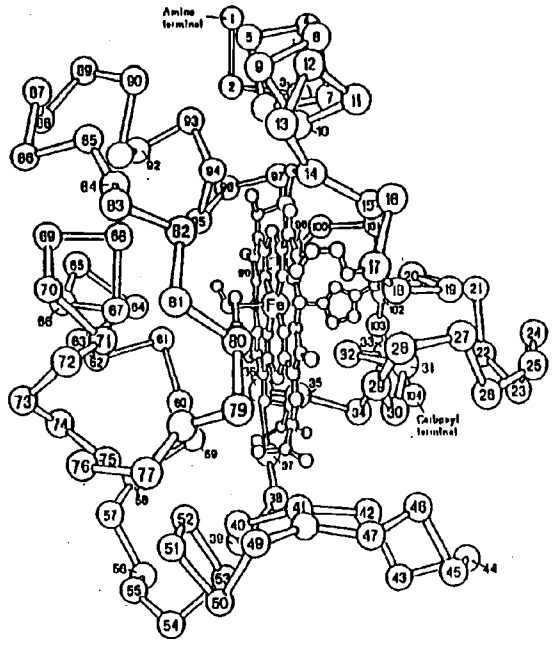

Fig.1

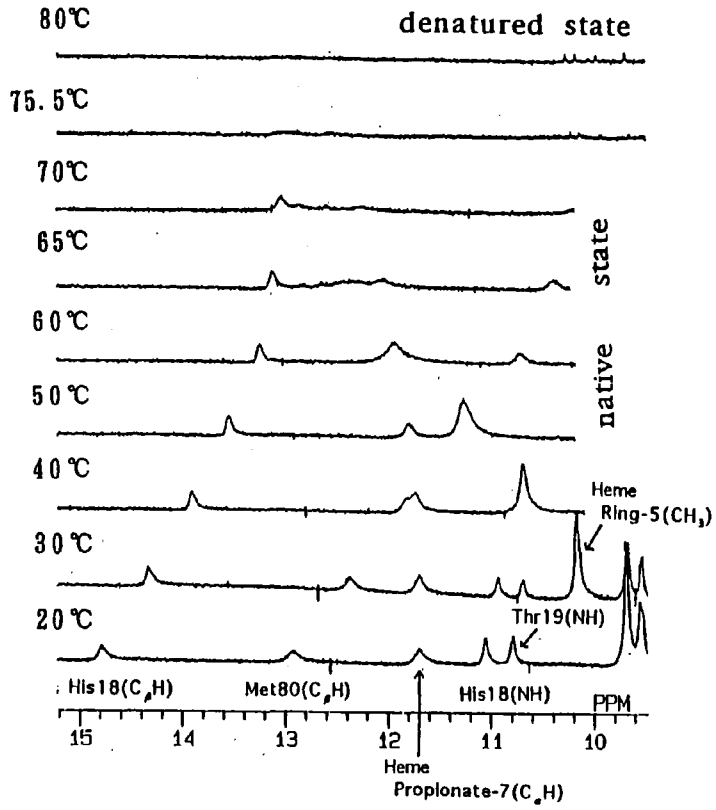

Fig.2