P–A4–33

TEMPERATURE DEPENDENCE OF PHOTOCYCLE KINETICS IN PHOTOACTIVE YELLOW PROTEIN AT LOW PH

VAN STOKKUM IHM,[1] GURAL J,[2] HOFF WD,[2,3] VAN BREDERODE ME,[1,2] HELLINGWERF KJ.[2]

[1]Fac. Phys. & Astron., Vrije Universiteit, Amsterdam (NL), [2]Dept. of Microbiol., Univ. of Amsterdam (NL), [3]Dept. Microbiol. & Mol. Genet., Univ. of Texas, Houston (USA)

Purpose: Elucidation of the thermodynamics of the last step of the photocycle, i.e. the return of the blue-shifted intermediate pB back to the ground-state pG of the photoactive yellow protein (PYP) from *E. halophila*. At pH below 3.5 PYP is protonated and converts to pB_{dark}, which is similar to pB.

Methods: Difference absorption traces, time gated and steady state absorption spectra were measured as a function of pH and temperature. The data were globally analysed using models for kinetics, spectra and thermodynamics of protein unfolding.

Results: The recovery pB→pG is decelerated by lowering the pH. At every pH the recovery accelerates and then decelerates upon increasing temperature. This can be described by a pH- and temperature-independent activation heat capacity change ($\Delta C_p^{\#}$) of -2.35 kJ/mol/K. From pG↔pB_{dark} equilibrium data a ΔC_p of 2.04 kJ/mol/K is estimated.

Conclusions: The formation of photocycle intermediate pB, and of the acid denatured state pB_{dark}, are accompanied by a heat capacity increase, which indicates partial unfolding of the photoreceptor.

P–A4–34

CRYSTAL STRUCTURE OF TRUNCATED INDOLE-3-GLYCEROL PHOSPHATE SYNTHASE FROM *S. SOLFATARICUS*: POSSIBLE FUNCTION OF THE N-TERMINAL α_0-HELIX

KNÖCHEL TR,[1] HENNIG M,[1] DARIMONT D,[2] KIRSCHNER K,[2] JANSONIUS JN.[1]

Dept. of [1]Struct. Biol. and [2]Biophys. Chem., Biozentrum, Univ. of Basel (CH)

Indoleglycerol phosphate synthase (IGPS) has a common $(\beta/\alpha)_8$-barrel fold, but carries a N-terminal extension of about 50 residues. The first 24 of these residues form the helix α_0 that covers the active site cleft. The structure of a hyperthermostable monomeric IGPS form *Sulfolobus solfataricus* was recently solved at 2 Å resolution. A truncated variant, Δ(2-25)sIGPS, is 100 times less active, but unfolds reversibly in guanidinium hydrochloride, in contrast to the wild type enzyme. The X-ray structure of Δ(2-25)sIGPS has now been solved by molecular replacement at 2.8 Å resolution. A comparison with the structure of the native enzyme shows that the α_0-helix can be removed without significant effects on the structure of the TIM-barrel core. This may explain the unchanged thermostability of Δ(2-25)sIGPS with respect to sIGPS. We conclude that the role of the N-terminal extension is to stabilize additionally the structurally crowded transition state of the IGPS reaction.

P–A4–35

THE ACTIVATION ENTHALPIES OF H-D EXCHANGE REACTION FOR INDIVIDUAL AMIDE HYDROGENS IN LYSOZYME

YASUO NODA, YASUO FUKUDA, SHIN-ICHI SEGAWA

School of Science, Department of Physics, Kwansei Gakuin University, Nishinomiya, Japan

Purpose: The activation enthalpy is very important to elucidate the mechanism of structural fluctuations mediating the H-D exchange reaction of amide hydrogens. We determined it for individual amide hydrogens in lysozyme and lysozyme derivative with an extra cross-link.
Methods: Exchange behavior of amide hydrogens has been studied by observing the fingerprint region of COSY spectra. Hydrogen exchange reaction was carried out at pH 7.0 and several temperatures. The activation enthalpy for each amide hydrogens were determined.
Results: There exist 41 amide hydrogens whose exchange reactions are detectable. They are classified into two categories III and IV.
Conclusions: Category III hydrogens are exchanged through fluctuation with a low activation enthalpy(15-25kcal/mol). They are little affect by cross-linking. Category IV hydrogens are exchanged with a high activation enthalpy(50-80kcal/mol). They are drastically influenced by cross-linking. Further we found that the exchange rates of amide hydrogens of residues 34-39 (hinge region) are drastically retarded by cross-linking.

P–A4–36

ELECTROSTATIC CONTRIBUTION TO PROTEIN COMPLEX STABILITY

TAKAHASHI T.

Dept. of Life Sciences, Univ. of Tokyo (JAPAN)

Purpose: As organic molecules like proteins contain many ionizable residues, the assembly largely depends on pH and ionic strength. A calculation method to simulate the effects was developed and was applied to a protein molecule, ferritin, which contains 24 identical subunits.

Methods:
First, the effective dielectric constant of the ferritin molecule was evaluated by solving Poisson-Boltzmann equation numerically. Then, the ionization of the individual ionizable residues were determined based on Charge Monte Carlo simulation, which can be applicable to very large systems. The calculated total charge was compared with those by the exact statistical mechanics and the fast Tanford-Roxby algorithm.

Results: The surface electrostatic potential of ferritin molecules based on the calculated charge distribution agreed well with the measured one except for high pH region, where the molecule seems to be partially denatured. The surface potential inside the human H ferritin was positive. It was consistent with the ionizable residue distribution when Asp, Glu, His, Arg, and Lys were fully ionized. The simulated electrostatic free energy reproduced pH and ionic strength effect on the subunit interactions.

Conclusions: This method reproduced the experimental surface potential of ferritin molecules and also the protein oligomer stability dependence on pH and ionic strength.