A kinetics of bacteriorhodopsin reconstituted from three partial polypeptides indicates a new thermodynamic aspect of protein

Yutaka Tsujiuchi
Dept. of Chem., Akita U.
1-1 TegataGakuen-machi,Akita 010,Japan
phone:0188-33-5261(ext.2627) fax:0188-33-3049
e-mail:ucchan@quartet.ipc.akita-u.ac.jp

Bacteriorhodopsin (BR) is the sole protein constituent of the purple membrane of Halobacterium halobium, functions as a light-driven proton pump. A chromophoric retinal of BR is bound with lysine 216 residue of a single polypeptide of 26 kD via a protonated Schiff base linkage. The polypeptide consists of seven membrane-spanning alpha-helices and they are linked by extramembranous loops. At the first stage of light-driven proton pumping cycle the chromophore is the site of the primary events. Isomerization of retinal from all-trans to 13-cis occurs in the cycle and the Schiff base proton is released at an intermediate stage. As the result, a proton is translocated from the inside to the outside of the cell and electrochemical gradient across the membrane is produced. The cell uses the gradient for ATP synthesis and transports of ions and amino acids.

BR can be reconstituted from three partial polypeptides i.e. two individual helices (first helix and second helix) and the complementary five-helix fragment. Thermodynamical and photochemical properties of reconstituted bacteriorhodopsin (recBR) are different from native ones.

First, recBR has three kinds of thermal equilibrium depend on the temperature. Above 298 K recBR is in a thermal equilibrium between P560 and P470. P560 is the pigment which is stable at 298 K and has nearly the same photochemical properties as native protein. The numbers 560, 380 are relatively given as the wavelength [nm] at which these pigments most absorbs photons. P380 is the pigment which were not found in photo-reactions of native protein. Below 298 K recBR is in an another thermal equilibrium between P470 and P560. P470 were also newly found. At the temperature 298 K there is solely the pigment P560 which is nearly the same as native protein. Therefore 298 K is the transition temperature.

Second, recBR reacts at a very slow rate compared with native ones. From a kinetic study of M-intermediates-formation process from P560 to M405, it was found recBR changes its first photo-reaction rate 90 percent smaller around the photo-equilibrium state of which is occurred between P560 and K-intermediate K605. And it also indicates from the set of equations used for elucidation of experimental data that there are existed two different energy transfer level in the system.

These results indicate that the method taken in these thermodynamical and photochemical studies of recBR is expandable onto other proteins and is effective to predict the folding catalysis from secondary structure to tertiary structure of protein.