X-ray crystallographic study of bacteriorhodopsin's reaction intermediates at low temperature

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Bacteriorhodopsin (bR), the sole protein of the purple membrane of *Halobacterium salinarium*, functions as a light-driven proton pump. When the *trans* retinal in the light-adapted bR absorbs light, the protein undergoes a reaction cycle involving several intermediates (bR_{570} -> K_{590} -> L_{550} -> M_{412} -> N_{560} -> O_{640} -> bR_{570}). To investigate light-induced conformation changes of bR, we have previously prepared a well-ordered 3D crystal belonging to space group P622, and determined the structures of the ground state (bR_{570}) and the M intermediate at 2.5Å resolution. The result shows that formation of M is accompanied by a sliding movement of helix G towards the extracellur side.

To elucidate the driving force of this movement, we are challenging to determine structural changes induced in the earlier stage of the photocycle. Firstly we collected diffraction data from a photoequilibrium state between bR_{570} and K, which was established at 100K under green light illumination (λ=532nm). By comparing with the data of the ground state, we obtained information about the structural change in the primary photoreaction. In the course of this structural analysis, however, we have recognized that the bR molecule is very liable to X-ray damage. It is obvious that the true structural change induced by visible light can be determined only after carefully removing the effect of X-ray damage.

Figure 1 shows the time course of X-ray damage observed when the crystal at 100K was exposed to synchrotron X-ray radiation. A significant decrease in the electron density is seen at the side chain of Asp85 (a counter ion of the protonated Schiff base) and the nearby water molecule. From optical measurement of the crystal exposed to X-rays, it is shown that the retinal chromophore is chemically modified by high-energy photons; i.e. the absorption band at 580nm diminished and, instead, an absorption band with peaks at 510, 480 and 450nm appeared. Importantly this specific X-ray damage takes place within the exposure time that is necessary for collection of a full data set of X-ray diffraction. To minimize the effect of the X-ray damage, we have improved the procedure of data collection. Our recent structural analysis suggests that the retinal chromphore has a distorted *13-cis* configuration in the K state.

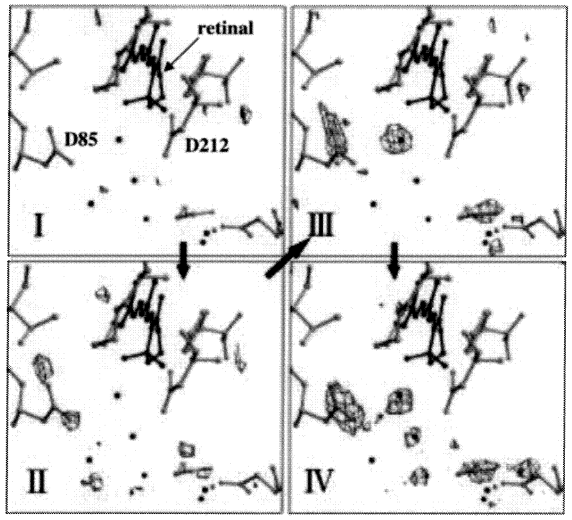

Fig.1 X-ray damage in the active site of bR_{570}

Subject title: **Structure determination of the catalytic region of the human complement component, C1r**

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The first component of the complement system (C1) plays a crucial role in the molecular immun responce. It initiates the classical pathway of complement activation, which results in the destruction of invading pathgens. C1 is a supramolecular complex, consisting of one C1q, two C1s, and two C1r molecules. C1r and C1s are modular serine proteases, which are responsible for the enzymatic activity of C1. The first enzymatic step during the classical pathway activation is the autoactivation of C1r, which involves the cleavage of an Arg-Ile bond in the serine protease domain. Activated C1r then cleaves the corresponding Arg-Ile bond in zymogen C1s. C1r and C1s form the Ca-dependent C1s-C1r-C1r-C1r-C1s tetramer, which associate with C1q to form the C1 complex. Up to now, there is very limited structural information concerning either the C1 complex or its subcomponents. C1r seems to be a key component of the complex since it makes a dimer forming the core of the $C1r_2C1s_2$ tetramer and it is capable of self activation. Our aim is to determine of the 3D-structure of the catalytic region of C1r. To meet this objective we crystallized the $(\gamma B)_2$ fragment of the enzyme. This fragment consist of three domains, two CCP and a serine protease module.

Molecular replacement is not an appropriate way to solve the structure since the homology of the available related structures is too low and the CCP domains are too small (the mol. mass of the dimeric fragment is ~90 kDa and that of an individual CCP is ~8 kDa.). Molecular replacement can only be reasonable for the serine protease domain. Therefore we prepared crystals with heavy atom derivatives. Due to the small size of our crystals it was impossible to test them using our home machine. In order to find the crystals of the best quality, in SPring-8 at BL41XU we had to start the experiments with testing of more than 25 crystals.

The following data sets were collected:

1. Native data set of C1r $(\gamma B)_2$ It was processed, resolution: 2.5 Å, spacegroup: $P2_1$, unit cell: 74.7, 160.6, 91.6, β=90.6°. R_{sym}=0.106
2. Native data set. res.: 2.8 Å, spacegroup: $P2_1$, unit cell: 74.8, 161.1, 91.2, β=90.7°
3. Due to lack of enough time we collected heavy atom derivative data sets at a constant 1.00 Å wavelength.
   PCMB (Hg) derivative: res.: 3.0 Å, spacegroup: $P2_1$, unit cell: 75.0, 161.4, 91.6, β=90.7°
4. Pt ($K_2Pt(CN)_4$ derivative: 3.1 Å, spacegroup: $P2_1$, unit cell: 74.7, 161.7, 91.6, β=90.8°
5. Sm ($Sm(OAc)_3$): 3.9 Å, spacegroup changed: $C2_1$, unit cell: 74.9, 165.9, 91.3, γ=90. 5?
6. Test crystal of an other serine protease bovine chymotrypsin, complexed with Ca^{++}: resolution 1.7 Å, spacegroup $P2_1$, unit cell: 64.3, 76.5, 84.3, β=107.7°.

Processing of heavy atom derivative data sets is under way.