Structural analysis during the photocycle of bacteriorhodopsin revealed by time resolved X-ray diffraction

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Introduction

Bacteriorhodopsin(BR) is the sole protein found in the purple membrane of *Halobacterium salinalium*. BR is folded into seven α helices spanning the lipid bilayer and an additional long segment in the aqueous region. The BR molecule form trimers and the trimers organized as a two-dimensional crystal in the purple membrane(PM). BR changes its structure during photocycle. Change in B and G helices are observed in M intermediate, while F and G helices change in N intemediate. In D96N-BR, MN intermediate is observed substitute for N intermediate. These structural change are revealed by static experiments. So we tested posibility of time resolved measurement of PM X-ray diffraction

Materials and Methods

The PM of wild type BR (WT) and D96N mutant BR (D96N) was purified. These two PMs were suspended by pH 7.0 or pH 8.5 buffers. Then PMs were oriented on mylar sheets, and incubated in constant humidity boxes of 86% r.h. or 75% r.h.. Time resolved X-ray diffraction were recorded at BL45XU-A for small angel X-ray scattering. Diffraction profiles were recorded with two dimensional area detector(Hamamatsu Photonics image intensifier and CCD camera). We measured photocycle of BRs at room temparature. A pulse Xenon flush lump were used to initiate the photocycle of BR.

Results and Discussions

We recorded diffraction data, 200 frames of 36ms or 109ms time resolution. We could see diffraction intensity decreasing caused by damage 70mA ring current mode. 20mA mode didn't cause damages on samples as 70mA mode. Because of intensity increase, although, we could see clearly the third element of structural change during M to N transition of WT-BR (pH8.5) or M to MN.transition of D96N (pH7.0, pH8.5). These data show structural change first occur before (or in) the M intermdiate, and second change occur during M to N transition (or M to MN transition). The second structural change is smaller than first one.