# Study on Reconstituted Bacteriorhodopsin(Abstracts of Doctral Dissertations)

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## ABSTRACT

INTRODUCTION

Bacteriorhodopsin (BR) is the sole protein costituent of the purple membrane of Halobacterium halobium, functions as a light-driven proton pump. A choromophoric retinal of BR is bound with lysine 216 residue of a single polypeptide of 26 kD via a protonated Schiff base linkage. 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 is a simple energy converter. Therefore, the molecular mechanism of the pump action has been the object of intensive studies. Site-directed mutagenesis is a recent popular technique to analise the roles of amino acid residues of proteins. To apply this technique, DNA encoding the protein has to be prepared and expressed in adequate systems. Khorana group constructed the DNA fragment encoding BR with codons which is suitable to E. coli but different from the native ones and expressed the protein. They made a lot of mutants of BR expressed in E. coli and have revieled several roles of amino acid residues for the functions.

In this paper we at first report the expression of BR gene in E. coli with the native codons and that we obtained the expressed proteins. Second we report the expression of partial genes of Bacteriorhodopsin encoding the helices ABCD and EFG. Third we report the photochemical properties of Bacteriorhodopsin reconstituted from two individual helices and the complementary five-helix fragment.

Chap.1 EXPRESSION OF BACTERIOOPSIN GENE IN ESCHERICHIA COLI

To explore the possibility of preparation of the partial peptide of Bacteriorhodopsin, the genes of Bacterioopsin (BOP) was expressed in Escherichia Coli. To express this we have constructed the inducible expression vector pUBO. The pUBO contain lac-promoter and on its downstream the segment of structure gene of lacZ and the gene of BOP. The expression of this fusion protein were detected by ELISA method using the polyclonal antibody raised against BR. The fusion protein obtained from E. coli in which had been transformed with the pUBO was estemated to be approximately 0.1% of the total protein of membrane fraction of

E. coli.

**Chap.2 EXPRESSION OF PARTIAL GENES OF THE BACTERIORHODOPSIN IN ESCHERICHIA COLI**

Partial genes of Bacterioopsin which correspond to ABCD helices and EFG helices of Bacteriorhodopsin were independently expressed in Escherichia Coli. To express them we have constructed the inducible expression vectors pUBOAIN, pUBOAIC, pKBOAIN, pKBOAIC, pTKBOAIN, pTKBOAIC. The vectors pUBOAIN, pKBOAIN, pTKBOAIN contain partial gene of BOP which corresponds to ABCD helices of BR , and the other vectors pUBOAIC , pKBOAIC , pTKBOAIC contain partial gene of BOP which corresponds to EFG helices of BR. The pUBOAIN and pUBOAIC contain lac-promoter and the partial genes of BR on its downstreem. The pKBOAIN and pKBOAIC contain taq-promoter and the partial genes of BR on its downstreem. The pTKBO-vectors contain the presequence of the manganese-stabilization protein of Anacystis Nidurans between lac-promoter and the partial genes of BR. The expression of these fusion proteins were detected by the way of ELISA method using the anti-BR serum. The fusion proteins prepared from E. coli which was transformed by the pTKBOAIN or pTKBOAIC were estemated to be more than one percent of the total protein of membrane fraction of E. coli.

**Chap.3 PHOTOCHEMICAL PROPERTIES OF BACTERIORHODOPSIN RECONSTITUTED FROM TWO INDIVIDUAL HELICES AND THE COMPLEMENTARY FIVE-HELIX FRAGMENT**

Low-temperature spectroscopy was used to examine the photochemical properties of Bacteriorhodopsin reconstituted from three fragments. At room temperature at pH 6.0, the recnstituted material shows essentially the same absorption spectrum as native BR, while upon raising the pH at room temperature or cooling the sample in glycerol, a second, blue-shifted peak appears. The pH and temperature dependence of the absorption spectrum indicates that the reconstituted BR is in a equilibrium between two pigments which we call P560 and P480. Both pigments convert to their own K intermidiates, which differ in absorption maxima, upon illumination with green light at -180 °C. Each K intermidiate can be reverted to its initial state by light. Similarly, both pigments convert to their own M intermidiates upon irrdiation with yellow light at -77 °C. The M intermidiate of both species can be reverted only to P560 by light. Both pigments are therefore photoactive. These unique photochemical properties of BR reconstituted from three fragments may be attributable to the lack of a covelent linkage in the loop connecting the A and B helices.

SUMMARY

This study is summarized. Some prospects are given.