Structure of Integral Membrane Proteins

W.A. Cramer
Department of Biological Sciences, Purdue University, West Lafayette, IN, U.S.A.

Introduction

During 2002, preliminary diffraction data were obtained on crystals of two different integral membrane proteins crystallized in detergent: (1) the 217-kDa cytochrome b_6f complex of oxygenic photosynthesis purified from thermophilic cyanobacteria and (2) a complex of the *E. coli* outer membrane receptor for vitamin B12, which is parasitized by certain colicins, with the receptor-binding domain of the colicins. The b_6f complex is involved in *trans*-membrane electron and proton transfer and in the transfer of lipophilic quinines from the membrane bilayer to serve as a hydrogen donor acceptor inside the cytochrome b_6f complex. The vitamin B12/colicin receptor, BtuB, serves as a model system for the study of protein (colicin) import into membranes.

Methods, Materials and Results

There were three sets of APS experiments on these two systems in 2002 at the APS. At beamline station 14-BM-C, on September 1 and October 24-26, 2002, low-resolution but promising data were obtained for both systems. Time was provided on SBC beamlines 19-BM and 19-ID on November 8-9, 2002. The experiments on beamline 19-BM resulted in a 2.75-Å data set. The structure could be solved by molecular replacement. Together with other experiments on the interaction of the colicin and the receptor, this structure showed a "fishing rod" complex of the 100-Å coiled-coil colicin E3 receptor-binding domain embedded in the BtuB receptor, from which it protrudes at a 40° oblique angle (Fig. 1).

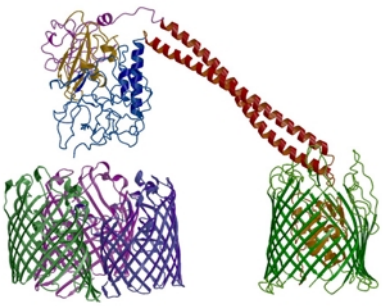

FIG. 1. Structure of colicin E3 molecule with elongate (100 Å) receptor binding domain complexed to vitamin B12 receptor (right) and close to interaction with second outer membrane protein required for cytotoxicity (NIH-GM-18457, Ref. 1).

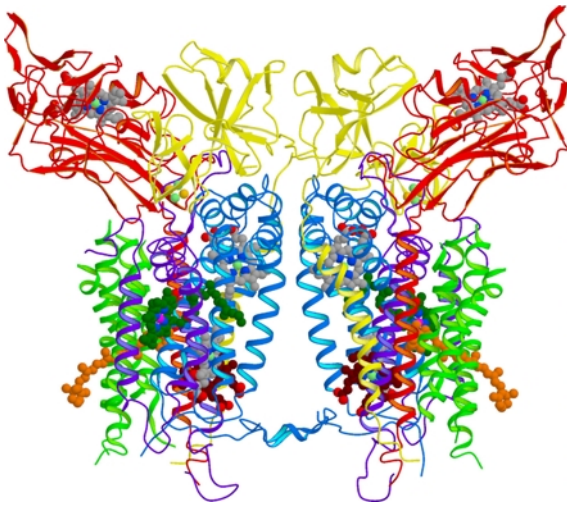

FIG. 2. Ribbon diagram describing structure of eight subunit dimeric MW = 217 kDa cytochrome b_6f complex of oxygenic photosynthesis, seen in cross-section (NIH GM-38323, Ref. 3).

Other data implied that the remainder of the colicin interacts with a second receptor in the outer membrane, thus forming a "translocon" for the cellular import of the colicin. These studies were published recently in *Nature Structural Biology* [2].

A 3.8-Å data set for the cytochrome *b*6*f* complex was obtained at SBC beamline 19-ID in the November 2002 experiments. This formed the basis for a draft of a manuscript for the *Proceedings of the National Academy of Science U.S.A.* on the unique methodology, "lipid augmentation," which had been developed for the crystallization of this membrane protein complex and was proposed to be applicable to other integral membrane proteins [1]. Subsequent experiments at Spring-8 and beamline SBC-19-ID in 2003 resulted in an improvement in the resolution to 3.4 and 3.0 Å, respectively, for native crystals and crystals made with the inhibitor tridecyl stigmatellin. A 3.0-Å structure of the cytochrome b_6f complex, showing eight subunits and 26 transmembrane helices in the dimeric structure (Fig. 2), has recently been published in *Science* [3], and, among other things, it completes the description of the structural architecture of oxygenic photosynthesis.

Acknowledgments

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References

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