THE ROLE OF PROTEIN PURIFICATION

IN THE STRUCTURE DETERMINATION OF AN INTEGRAL MEMBRANE PROTEIN. G. McDermott1, S.M. Prince, M. Pain2, P. Barford3 Depart. of Biochemistry and Biophysics, UCSD, La Jolla, CA 92037.

Large amounts of the porin from Rhodobacter capsulatus have been expressed in 1990 for Rhodobacteria at the University of Illinois at Urbana-Champaign and the University of Chicago. A native porin with a diameter of about 1 nm. Three pore between the walls of the B800 and B850. The Mg ligands for the B850 BCAs are α-His34 and β-His35 as expected from comparison with the Rps. acidiphila structure, but the Mg of the B800 BCA is bound to α-Asp6 and to a histidine or a methionine. The Q˙ transition dipole moments of neighboring B850 and B800 BCAs are nearly parallel to each other, which is optimal for efficient Förster excitation transfer. B850 BCA and one of the two B850 BCAs are involved in an edge to edge contact with lycopene, thus Dexter mechanism can be functional for energy transfer from lycopene to BCAs.

Crystallography of Biological Macromolecules

Peptides that form α-helices with a strongly amphipathic nature are capable of solubilizing membrane proteins if they fulfill certain criteria. Adequate length, a flat hydrophobic surface, and a polar exterior are the main components of these peptides. Variations in the initial peptide structure have been made and resulted in many variations on the original theme. The crystal structure of the initial peptide used to solubilize membrane proteins is described and shows the nature of the interface between hydrophobic surfaces and laterally between adjacent membrane-spanning peptides. The crystal structure was solved entirely from α-helical models using molecular replacement.

CRYSTALLIZATION OF AN 80 KD OUTER MEMBRANE PROTEIN. Susan Buchanan1, Barbara Smith1, Lalitha Venkatramani2, Dick van der Helm3, and Johann Deisenhofer4. Howard Hughes Medical Institute, UT Southwestern Medical Center, 5323 Harry Hines Blvd. Dallas, TX 75335-9050. Department of Chemistry and Biochemistry, University of Oklahoma, Norman, OK 730192

Ferric enterobactin receptor (FepA), an outer membrane protein from Escherichia coli, has been overexpressed to produce large quantities of insoluble cytoplasmic inclusion bodies. The inclusion bodies have been solubilized in urea and refolded using a combination of stabilizing agents. The refolded protein was subsequently purified by gel filtration chromatography. The crystal structure was solved entirely from α-helical models using molecular replacement.

Refolded FepA was crystallized according to methods developed for native (membrane-inserted FepA; the resulting crystals have the identical space group and cell dimensions determined for native FepA crystals. A low temperature native data set has been collected to 2.9 Å resolution and a search for heavy atom derivatization is in progress, using crystals from both native and refolded sources. Current yields from the inclusion body expression system are approximately 10 mg/l, making this method suitable for structural studies of other outer membrane proteins.

Membrane Proteins II

STRUCTURE-FUNCTION RELATIONSHIPS IN THE MEMBRANE CHANNEL PORIN. Georg E. Schulz, Institut für Organische Chemie und Biochemie Albertstra. 21, 79104 Freiburg im Breisgau, Germany

Porins form channels in the protective outer membrane of Gram-negative bacteria that are permeable for polar molecules, but discriminative against nonpolar ones. The first crystal structure had been reported in 1990 for Rhodobacter capsulatus [1]. All structurally known porins have subunits with 16- or 18-stranded β-barrels surrounding a pore with a diameter of about 1 nm. Three barrels associate along their axes to form a trimer [2-6]. All porins contain two girdles of aromatic residues facing the membrane at its two polar-nonpolar borders, which are likely to fulfill a shielding function. Moreover, all general pores are lined by ionogenic groups that segregate into negatively and positively charged rims. It is suggested that these constitute an electric separator testing solute solubility [6-8]. In two porins which had been classified as unspecific, we detected ligand binding sites.

Large amounts of the porin from R. blasticus were expressed in inclusion bodies in E. coli and recovered to form crystals iden-