4.2. Crystallization of membrane proteins

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4.2.1. Introduction

At the time of writing, the Protein Data Bank contains more than 8000 entries of protein structures. These belong to roughly 1200 sequence-unrelated protein families, which can be classified into ~350 different folds (Gerstein, 1998). However, all known membrane-protein structures belong to one of a dozen membrane-protein families. Membrane proteins for which the structures have been determined are bacterial photosynthetic reaction centres, porins and other β-strand barrel-forming proteins from the outer membrane of Gram-negative bacteria, bacterial light-harvesting complexes, bacterial and mitochondrial cytochrome *c* oxidase, the cytochrome bc_1 complex from mammalian mitochondria, two cyclooxygenases, squalene cyclase, and two bacterial channel proteins. These structures have been determined by X-ray crystallography. In addition, the structures of two membrane proteins, namely that of bacteriorhodopsin and that of the plant light-harvesting complex II, have been solved by electron crystallography (see Chapter 19.6). Table 4.2.1.1 provides a list of the membrane proteins with known structures. It also contains the key references for the structure descriptions and the crystallization conditions.

It is known from genome sequencing projects that 20–35% of all proteins contain at least one transmembrane segment (Gerstein, 1998), as deduced from the occurrence of stretches of hydrophobic amino acids that are long enough to span the membrane in a helical manner. These numbers may be an underestimate, because β-strand-rich membrane proteins like the porins, or membrane proteins which are only inserted into the membrane, but do not span it ('monotopic membrane proteins'), like cyclooxygenases (prostaglandin-H synthases), cannot be recognized as being membrane proteins by inspecting their amino-acid sequences.

Why do we know so few membrane-protein structures? The first reason is the lack of sufficient amounts of biochemically well characterized, homogeneous and stable membrane-protein preparations. This is especially true for eukaryotic receptors and transporters (we do not know the structures of any of these proteins). For these, a major problem is the lack of efficient expression systems for heterologous membrane-protein production. It is therefore not surprising that most membrane proteins with known structure are either involved in photosynthesis or bioenergetics (they are relatively abundant), or originate from bacterial outer membranes (they are exceptionally stable and can be overproduced). Second, membrane proteins are integrated into membranes. They have two polar surface regions on opposite sides (where they are in contact with the aqueous phases and the polar head groups of the membrane lipids) which are separated by a hydrophobic belt. The latter is in contact with the alkyl chains of the lipids. As a result of this amphipathic nature of their surface, membrane proteins are not soluble in either aqueous or organic solvents. To isolate membrane proteins one first has to prepare the membranes, and then solubilize the membrane proteins by adding an excess of detergent. Detergents consist of a polar or charged head group and a hydrophobic tail. Above a certain concentration, the so-called *critical micellar concentration* (CMC), detergents form *micelles* by association of their hydrophobic tails. These micelles take up lipids. Detergents also bind to the hydrophobic surface of membrane proteins with their hydrophobic tails and form a ring-like detergent micelle surrounding the membrane protein, thus shielding the hydrophobic belt-like surface of the membrane protein from contact with water. This is the reason for their ability to solubilize membrane proteins, although with detergents with large polar head groups it is sometimes difficult to achieve a rapid and complete solubilization. The solubilizate, consisting of these mixed protein–detergent complexes as well as lipid-containing and pure detergent micelles, is then subjected to similar purification procedures as are soluble proteins. Of course, the presence of detergents complicates the purification procedures. The choice of the detergent is critical. The detergent micelles have to replace, and to mimic, the lipid bilayer as perfectly as possible, in order to maintain the stability and activity of the solubilized membrane protein. The solubilization of membrane proteins has been reviewed by Hjelmeland (1990) and the general properties of the detergents used has been reviewed by Neugebauer (1990).

4.2.2. Principles of membrane-protein crystallization

There are two principal types of membrane-protein crystals (Michel, 1983). First, one can think of forming two-dimensional crystals in the planes of the membrane and stacking these two-dimensional crystals in an ordered way with respect to up and down orientation, rotation and translation. This membrane-protein crystal type ('type I') is attractive, because it contains the membrane proteins in their native environment. It should even be possible to study lipid–protein interactions. Crystals of bacteriorhodopsin of this type have been obtained either upon slow removal of the detergent by dialysis at high ionic strength (Henderson & Shotton, 1980), or by a novel approach using lipidic bicontinuous cubic phases (Landau & Rosenbusch, 1996; Pebay-Peyroula *et al.*, 1997; see also below). Alternatively, one can try to crystallize the membrane protein with the detergents still bound in a micellar manner. These crystals are held together *via* polar interactions between the polar surfaces of the membrane proteins. The detergent plays a more passive, but still critical, role. Such 'type II' crystals look very much like crystals of soluble globular proteins. The same crystallization methods and equipment as for soluble globular proteins (see Chapter 4.1) can be used. However, the use of hanging drops is sometimes difficult, because the presence of detergents leads to a lower surface tension of the protein solution. Intermediate forms between type I and type II crystals are feasible, *e.g.* by fusion of detergent micelles.

The use of detergent concentrations just above the CMC of the respective detergent is recommended in order to prevent complications caused by pure detergent micelles. Unfortunately, the CMC is not constant. Normally, the CMC provided by the vendor has been determined in water at room temperature. A compilation of potentially useful detergents, their CMCs and their molecular weights is presented in Table 4.2.2.1. The CMC is generally lower at high ionic strength and at high temperatures. The presence of glycerol and similar compounds, as well as that of chaotropic agents (Midura & Yanagishita, 1995), also influences (decreases) the CMC.

4.2.3. General properties of detergents relevant to membrane-protein crystallization

The presence of detergents sometimes causes problems. The monomeric detergent itself can crystallize, *e.g.* dodecyl-β-D-maltoside at 4 °C in the presence of polyethylene glycol. The detergent crystals might be mistaken for protein crystals. Detergent micelles possess attractive interactions (see Zulauf, 1991). Upon addition of salts or polyethylene glycol, or upon temperature changes, a phase separation may be observed: owing to an increase in these attractive interactions, the detergent micelles 'precipitate', forming a viscous detergent-rich phase and a detergent-depleted

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