Membrane Protein Crystallization at the Advanced Light Source

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INTRODUCTION

Proteins are ubiquitous in Nature. Typically, they function as the principle components and/or the controlling agents of biological systems. All protein molecules are comprised of one or more chains of polymerized amino acids. As such, these linear chains must 'fold' into particular spatial conformations to be fully functional [1]. Constructing an accurate model of a protein's molecular topology is an essential step on the path towards understanding its *in vivo* function [2]. In recent years, advances in the technology of gene cloning and sequencing have resulted in an exponential increase in the number of identified genes; however, understanding the functions of these genes products at the molecular level has not kept pace [3]. Although it is possible to determine the amino acid sequence of a protein given the DNA sequence, it is not possible to predict how the protein will fold: this can only be determined experimentally. For some years now, the technique that has been most successfully used to obtain this information is protein X-ray crystallography [3].

In recent years, the crystallization of proteins has moved from being the exclusive domain of the specialist crystallographer to become a routine practice in most laboratories that study protein biochemistry [4]. Several thousand proteins have now been crystallized and their corresponding crystallographic structures established. In fact, the technique has now evolved to the extent that a new protein structure is currently being deposited in the protein structure databank every five hours. In spite of this fact, one class of biologically important proteins remains underrepresented in terms of available structural information: The molecular structure of only a few integral membrane proteins has been established. Primarily, this paucity of structural information can be attributed to the particular biochemical and biophysical properties of membrane proteins [5].

MEMBRANE PROTEINS

Integral membrane proteins are proteins that are vectorially inserted into the lipid bilayer of biological membranes [6]. A typical membrane protein has three topologically distinct domains: two hydrophilic extra-membranous domains and the hydrophobic domain that spans the lipid bilayer. *In vitro,* it is possible to 'release' membrane proteins into solution by treating membranes with suitable surfactants, such as non-ionic detergents [6]. However, the strong amphipathic character of the resultant molecule makes it insoluble in both polar and apolar solvents. To render the protein soluble in aqueous solution, the hydrophobic region must be covered with a 'jacket' that interacts readily with the bulk solute [7]. In practice, this is achieved by constantly maintaining the protein in a solution containing detergent at a concentration above its critical micelle concentration. Under these conditions the protein molecules are incorporated into detergent micelles, and hence present a mostly hydrophilic surface to the solvent [7].

CRYSTALLIZATION OF MEMBRANE PROTEINS: BACKGROUND

Proteins crystallize according to the principles that govern the crystallization of simple salts; i.e. by making a solution of the protein supersaturated, nucleation and crystal growth may occur [8]. In practice, the method used to crystallize proteins takes advantage of the way protein solubility varies as a function of ionic strength: for example, adding increasing amounts of ammonium sulfate will

cause most protein solutions to become saturated, and if the conditions are favorable, supersaturated. Unfortunately, increasing ionic strength of a membrane protein solution can cause the detergent to partition into a separate phase. When this occurs, the protein rapidly migrates into the detergent enriched phase, where depletion of the protein's solvation water results in its rapid denaturation [7].

To overcome this problem, Michel pioneered a method of employing 'amphiphilic additives' to prevent detergent phase separation. This allowed membrane proteins to be crystallized in a manner similar to globular proteins [7]. This was a landmark event in the history of X-ray crystallography. Accordingly, for this feat Michel, Deisenhofer and Huber were awarded the Nobel prize in 1988. Unfortunately, optimism that this work would result in the elucidation of many more membrane protein structures proved short-lived.

Protein crystallization is fundamentally an empirical process. In most instances, a set of parameters successfully used to crystallize one protein will not be applicable to other proteins. *A priori* there is no way of formulating such conditions; they must be determined experimentally for each protein [8]. It was found that, in addition to the other parameters that can affect the success of crystallization experiment, the choice of detergent and additive is crucial [9]. Hence, for membrane proteins the number of parameters that must be permuted is much greater than for soluble globular proteins, which has the effect that typically many more experiments must be carried out to establish the conditions that lead to the crystallization of a membrane protein. This exacerbates yet further an inherent problem of membrane protein crystallization, the generally limited availability of starting material. Most membrane proteins are only present *in vivo* in small quantities, making purification directly from tissue impractical. Unfortunately, when membrane proteins are overexpressed in heterologous expression systems, protein yields tend to be comparatively low [10]. This obviously restricts the number of crystallization trials that can be conducted, and consequently reduces the likelihood of ever obtaining crystals.

PROTEIN CRYSTALLIZATION AT THE ALS

This prompts the question: why is the ALS an ideal place to conduct membrane protein crystallization trails? The answer lies in the fact that en route to obtaining 'good' quality crystals one often goes through various stages of getting poor quality ones; i.e. crystals whose internal order is such that they only diffract X-rays to low resolution. Although these can't be used for data collection, information on their diffraction properties can be used in the iterative process of optimizing the crystal growth conditions [11]. In this context, it is essential to use a highly collimated, intense synchrotron beam, such as beamline 5.0.2 at the ALS. Having ready access to such a facility means that we are able to get rapid and quantifiable feedback on the effect protocol changes have on the resultant crystal's diffraction properties, and hence greatly speed up the process of optimizing diffraction and minimize the amount of protein used in the process. In addition, by testing 'sub-optimal' crystals, such as microcrystals (i.e. those with dimensions of less than 50 μm) we are stretching the limits of the data collection facilities on BL 5.0.2. By doing so, the data collection capabilities of this beamline will, as a matter of course, be further developed and enhanced.

CURRENT PROJECTS

The proteins we are currently crystallizing are either integral membrane proteins or proteins which interact with membrane proteins. In particular we are interested in proteins involved in process of signal transduction across biological membranes. In general, the components of these systems

include integral membrane proteins such as receptors or ion channels, as well as extracellular ligands such as hormones or cytokines and intracellular effectors like kinases. A particular focus of the laboratory is the erythropoietin system. Erythropoietin is a cytokine involved in the proliferation and differentiation of the erythroid progenitor cells that eventually form red blood cells [12]. Its effects are transmitted to the interior of the cell via the erythropoietin receptor and a number of downstream effectors such as the kinase JAK2. We are currently conducting crystallization trials using erythropoietin receptor, and are beginning to conduct trials with JAK2.

Other projects in the lab, which are at various stages along the pathway from isolating the gene to conducting crystallization trials, include the membrane protein PMP-22, which is involved in several hereditary peripheral nervous system neuropathies [13], and MOMP, the Chlamydia surface antigen, whose crystallographic structure may hold the key to a successful vaccine against the world's most common sexually transmitted disease [14].

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