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ScienceDirect
Journal of Structural Biology 160 (2007) 313–323
Journal of Structural Biology
www.elsevier.com/locate/yjsbi

7 Å projection map of the S-layer protein sbpA obtained with trehalose-embedded monolayer crystals

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Received 19 March 2007; received in revised form 17 May 2007; accepted 5 June 2007
Available online 15 June 2007

Abstract

Two-dimensional crystallization on lipid monolayers is a versatile tool to obtain structural information of proteins by electron microscopy. An inherent problem with this approach is to prepare samples in a way that preserves the crystalline order of the protein array and produces specimens that are sufficiently flat for high-resolution data collection at high tilt angles. As a test specimen to optimize the preparation of lipid monolayer crystals for electron microscopy imaging, we used the S-layer protein sbpA, a protein with potential for designing arrays of both biological and inorganic materials with engineered properties for a variety of nanotechnology applications. Sugar embedding is currently considered the best method to prepare two-dimensional crystals of membrane proteins reconstituted into lipid bilayers. We found that using a loop to transfer lipid monolayer crystals to an electron microscopy grid followed by embedding in trehalose and quick-freezing in liquid ethane also yielded the highest resolution images for sbpA lipid monolayer crystals. Using images of specimens prepared in this way we could calculate a projection map of sbpA at 7 Å resolution, one of the highest resolution projection structures obtained with lipid monolayer crystals to date.

Keywords: Electron crystallography; S-layer protein; Lipid monolayers; Sugar embedding

1. Introduction

Fromherz was the first to demonstrate that soluble proteins can form ordered arrays on the surface of a lipid monolayer at the air/water interface and that these arrays can be imaged by electron microscopy (EM) (Fromherz, 1971). Subsequently, the lipid monolayer crystallization method was used to make soluble proteins amenable to structural studies by electron crystallography (Uzgiris and Kornberg, 1983). The underlying principle of array formation is that association of a target protein with the lipid monolayer leads to concentration and partial alignment of the protein. Since the lipid monolayer is in its fluid phase, the lipids, and hence the associated proteins, can diffuse in the plane of the monolayer, allowing the proteins to interact with each other and under favorable conditions to form regular arrays. The lipid monolayer is usually formed with neutral lipids spiked either with charged lipids to induce association of proteins by electrostatic interactions (Darst et al., 1988; Mosser et al., 1991; Taylor and Taylor, 1993, 1999) or with functionalized lipids, such as lipids containing Ni–NTA headgroups that specifically recruit His-tagged proteins to the monolayer (Kubalek et al., 1994). Once introduced, the method was subsequently adapted for use with membrane proteins (Lévy et al., 1999; Lebeau et al., 2001) and for the assembly of protein complexes (Celia et al., 1999; Kelly and Taylor, 2005; Kelly et al., 2006).

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1047-8477/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.jsb.2007.06.002

Although it was possible to record high-resolution (~3 Å) EM data of streptavidin crystals on a lipid monolayer (Kubalek et al., 1991; Avila-Sakar and Chiu, 1996), a persistent problem in the use of this technique lies in the transfer of lipid monolayer crystals to an EM grid without deteriorating the order of the protein arrays. In addition, lipid monolayer samples are often not flat, and to date no high-resolution images have been reported for tilted lipid monolayer crystals. A reliable protocol to reproducibly prepare specimens for high-resolution EM data collection would thus have the potential to boost interest in lipid monolayer crystallization for structural studies of soluble proteins.

Once crystals have formed on the lipid monolayer, often using a Teflon crystallization block (Fig. 1a), they can be transferred to an EM grid using the direct transfer method (Fig. 1b and c), historically referred to as the Langmuir–Schaefer transfer method (Langmuir and Schaefer, 1938). For this technique, a hydrophobic carbon film mounted on an EM grid is brought into direct contact with the hydrophobic tails of the lipids in the monolayer (Fig. 1b). When the grid is lifted off, the monolayer and hence the associated crystals adhere to the carbon film and are thus transferred to the grid (Fig. 1c). The crystals on the grid can then be blotted and either negatively stained or vitrified for subsequent observation in the electron microscope. However, touching a lipid monolayer with a carbon film imparts mechanical force that tends to distort the attached crystals (Brisson et al., 1999). Thus, a holey grid is often used in conjunction with direct transfer and specimen vitrification. Images are then taken in regions without carbon film, where the crystals should be least affected. Although variations of this technique have yielded both the highest resolution lipid monolayer crystals to date (Kubalek et al., 1991; Avila-Sakar and Chiu, 1996) as well as three-dimensional (3D) reconstructions at lower resolutions (Tang et al., 2001; Wendt et al., 2001; Liu et al., 2003, 2004, 2006; Kelly et al., 2006), high-resolution 3D structures have proved difficult to obtain in this manner. An alternative approach to the direct transfer method is the loop transfer technique, in which a wire loop is used to lift off the lipid monolayer and to deposit it on an EM grid (Fig. 1d and e). This method was initially developed with holey carbon grids (Asturias and Kornberg, 1995), but a continuous carbon support film can also be used. In contrast to the direct method, when loop transfer is used, the protein array rather than the lipid monolayer makes contact with the carbon film (Fig. 1e), leading to a more robust interaction between the protein crystal and the carbon support film.

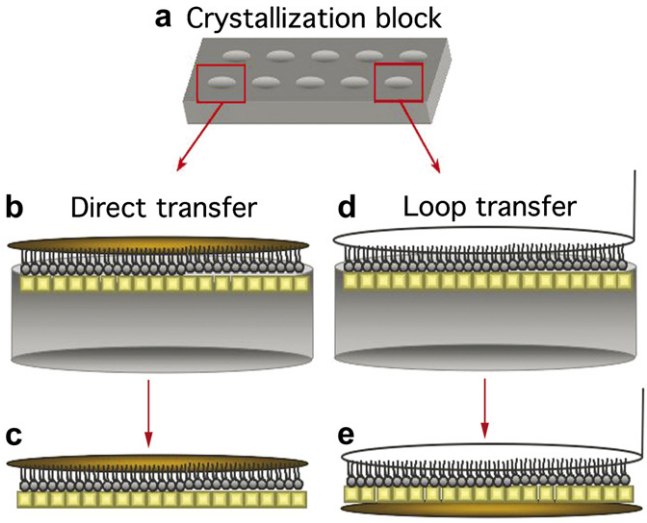

Fig. 1. The direct and loop transfer methods. (a) Teflon block used to grow 2D crystals on lipid monolayers. (b, c) Direct transfer method, after which the lipid monolayer is in contact with the carbon film. (d, e) Loop transfer method, after which the protein crystal is in contact with the carbon film. For (b–e) protein crystals are in yellow while the EM grid is colored gold.

In a related field, electron crystallography of conventional two-dimensional (2D) crystals, i.e., membrane proteins reconstituted into lipid bilayers, a number of high-resolution structures have now been determined (Henderson et al., 1990; Kühlbrandt et al., 1994; Kimura et al., 1997; Murata et al., 2000; Gonen et al., 2005; Hiroaki et al., 2006; Holm et al., 2006). Advances in specimen preparation, in particular the embedding of the specimen in sugar solutions (Unwin and Henderson, 1975; Jap et al., 1990; Wang and Kühlbrandt, 1991; Hirai et al., 1999; Gyobu et al., 2004), have played a crucial role in the success of this method. It was thus of interest to us, whether sugar embedding could be adapted to the preparation of lipid monolayer crystals and how it would compare to the conventional preparation methods currently used for lipid monolayer crystals. As a test specimen to study specimen preparation methods, we selected the S-layer protein, sbpA. S-layer proteins have been historically used for electron crystallographic studies due to their inherent propensity to self-assemble into ordered arrays (for a review, see Baumeister et al., 1988). In particular, we chose the protein sbpA from *Bacillus sphaericus*, because it is easy to purify from the cell wall of native bacteria (Schuster et al., 2005) and it has been shown to form 2D crystals (Pum and Sleytr, 1994) in a calcium-dependent manner (Pum and Sleytr, 1995). Native and recombinant S-layer proteins have also recently been used as building blocks to design 2D scaffolds with engineered properties for various nanotechnology applications (Sleytr et al., 2003), raising interest in determining their structure. However, structural studies of sbpA crystals have so far not exceeded a resolution of about 20 Å (Lepault and Pitt, 1984; Lepault et al., 1986). By systematically testing various specimen preparation protocols, we found that we could produce the best specimens by transferring the lipid monolayer crystals to a continuous carbon film using loop transfer followed by embedding the sample in trehalose and quick-freezing in liquid ethane. With images of specimens prepared in this way, we could calculate a projection map of sbpA at a resolution of 7 Å. This is one of the highest resolution maps obtained with 2D crystals grown on lipid monolayers to date. At this point the resolution may no longer be limited