

Short crystallization paper

# Crystallization of bacteriorhodopsin solubilized by a tripod amphiphile

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Received 21 March 2005; received in revised form 22 April 2005; accepted 26 April 2005

Available online 23 May 2005

## Abstract

Bacteriorhodopsin (bR) is solubilized efficiently as a monomer by a novel surfactant, a tripod amphiphile (TPA), which permits the formation of purple hexagonal bR crystals under several conditions. The crystals, although small, diffract to 2.5 Å resolution using synchrotron radiation. TPA may be useful for the solubilization, purification, and crystallization of other membrane proteins.

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**Keywords:** Membrane protein; Crystallization; Bacteriorhodopsin; Tripod amphiphile; Detergent; Surfactant

## 1. Introduction

Integral membrane proteins account for up to one third of the genome in different organisms [1]. Despite the importance of membrane proteins in cell physiology, there is a paucity of high-resolution (i.e., 3 Å or better) membrane protein structures. This is in large part attributable to difficulties in obtaining well diffracting crystals of membrane proteins that are amenable to X-ray crystallographic structure determination [2–5]. In order to prepare such crystals, membrane proteins typically must be purified to form monodisperse solutions by first using detergents that extract membrane proteins from their native heterogeneous membrane bilayer environments [6]. Bacteriorhodopsin in purple membranes is an exception in that crystals that diffract to 2.8 Å can be grown in the lipidic cubic phase [7]. Detergents associate around the hydrophobic surfaces of membrane proteins to form protein–detergent complexes. Unfortunately, these protein–detergent complexes are notoriously unstable; the protein often denatures in the detergent environment [8,9]. Hence, there have been significant

efforts to design novel detergents that can form a stable the protein–detergent complex during protein purification and crystallization [10–15]. In general, increased stabilization of the protein–detergent complex is achieved by effectively coating more of the surface area of the membrane protein with the novel detergent molecules [4,16,17]. However, there is no report on using any of these novel detergents for membrane protein crystallization. In a membrane protein crystal, the creation of ordered contacts is a prerequisite for formation of the three-dimensional lattice [18]. Hence, there can be a dichotomy for the role of detergent interactions: the detergent needs to form a stable protein–detergent complex while being flexible enough to permit formation of protein–detergent complex contacts in a crystal [19].

The light-harvesting protein bacteriorhodopsin (bR) is a well-characterized model system used in developing methods for membrane proteins. In previous studies, three-dimensional crystals of bR have been obtained under a variety of conditions, including in the detergent-solubilized state [20–23]. Most of these conditions required the incorporation of bacteriorhodopsin into lipidic cubic phases, vesicles, or bicelles, which are not standard detergent micelle phases. The process of crystallization usually requires that the membrane proteins be purified

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using detergents and then incorporated into these phases. Earlier reported crystals of detergent-solubilized bR diffracted anisotropically and to low resolution [24,25]. The 2.9 Å resolution bR structure using synchrotron radiation has been determined from crystals grown from n-octyl  $\beta$ -D-glucopyranoside solubilized bR [26]. However, bR solubilized in  $\beta$ -OG is very light sensitive and requires special conditions for crystallization.

We test the use of a tripod amphiphile for the crystallization of bacteriorhodopsin. Tripod amphiphiles (TPAs) are surfactants with hydrophobic portions that are believed to be more rigid, and therefore less conformationally heterogeneous, than other detergents [27]. A tripod amphiphile (Fig. 1) has previously shown to be an excellent agent for solubilization and stabilization of bR [12]. While many detergents can be used to solubilize bR, only a subset allow crystallization of the protein, and even fewer yield crystals of high diffraction quality [25]. Here, we test whether TPA can support the formation of bR crystals. The TPA class of surfactants may promote ordered contacts between protein molecules, leading to a greater success rate in crystallization.

## 2. Materials and methods

Purple membrane was isolated from *Halobacterium salinarum* [28]. TPA was synthesized as previously described [12]. Bacteriorhodopsin was extracted from membranes by using 18 mM TPA as previously described [12]. Solubilized bR was purified using a Superdex 200 HR column. The intensely purple-colored bR (in 20 mM Na/KPO<sub>4</sub>, pH 5.5, 100 mM NaCl, 18 mM TPA) was concentrated to 8 mg/mL and screened for crystallization using hanging drop vapor diffusion using 1  $\mu$ L protein with 1  $\mu$ L precipitant at 4 °C with ambient light present. Initial crystallization screening was conducted with the Crystal Screen, Crystal Screen 2, PEG/Ion Screen, and MembFac solutions from Hampton Research and JBScreen1 and JBScreen2 from Jena Biosciences. Several conditions produced clusters of small, purple-colored crystals. Grids were setup around initial hits to optimize bR crystallization. Crystals were moved to matching buffer containing 30% v/v glycerol and flash-cooled in liquid nitrogen. Cryoprotected crystals were exposed to X-rays with a wavelength of 1.0 Å,

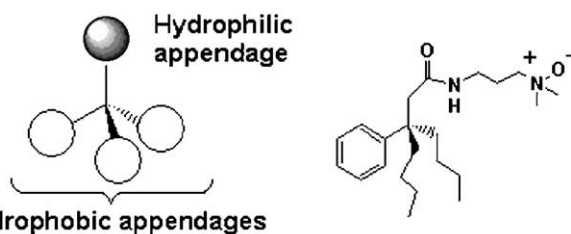


Fig. 1. A schematic and chemical structure of the tripod amphiphile used in this study.

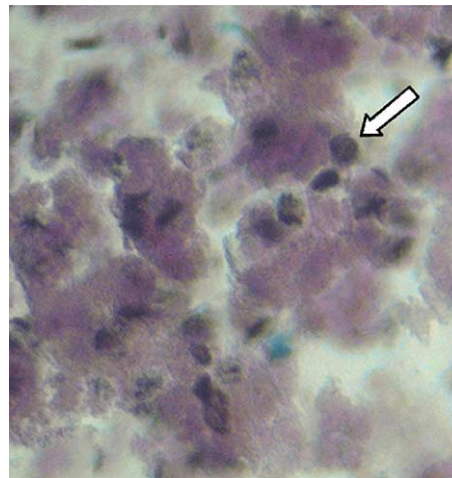


Fig. 2. Crystals of bacteriorhodopsin grown in the presence of TPA. The crystals are purple hexagonal plates. The arrow indicates a typical bR crystal.

at Beamline 17-ID of the Advanced Photon Source in Argonne, IL.

## 3. Results and discussion

bR has been shown to be solubilized in TPA with a yield that is better than n-octyl  $\beta$ -D-glucopyranoside, Triton X100, and n-dodecyl  $\beta$ -D-maltopyranoside [12]. The TPA-solubilized-bR has an optical spectrum characteristic of a monomer [12,24]. Surprisingly, TPA-solubilized bR is more stable to light than bR solubilized in the aforementioned detergents. During the set-up of crystallization trials using pipettors, there were no detectable changes in the solution viscosity of the TPA solubilized bR solution. An increase of such solution viscosity is indicative of significant increase of detergent concentration that can occur during the process of protein concentration.

Crystals of bR/TPA reaching sizes of approximately 20 $\times$ 40 $\times$ 20  $\mu$ m after 5 days at 4 °C appeared under several conditions: 2 M ammonium sulfate, 2% v/v PEG400, 0.1 M

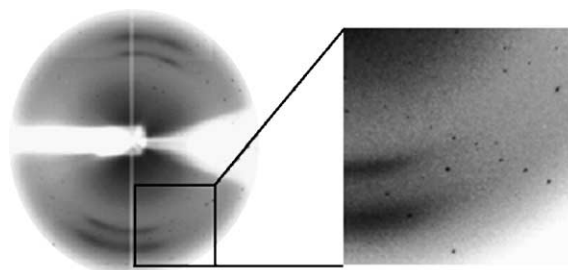


Fig. 3. Diffraction from bR crystals grown in TPA. The sample was oscillated 1° during a 15-s exposure. At 250 mm from crystal to detector, the edge corresponds to 2.5 Å. A region showing regular spacing of reflections has been enlarged.

HEPES, pH 7.5; 30% w/v PEG8000, 0.2 M ammonium sulfate; and 1 M ammonium phosphate, 0.1 M lithium sulfate, 0.1 M sodium acetate, pH 4.6. The best-diffracting specimens (Fig. 2) were those grown from 2 M ammonium sulfate, 2% v/v PEG400, 0.1 M HEPES, pH 7.5 and 18 mM TPA. These solution crystallization conditions are distinctly different from the conditions used for the crystallization of bR in solution, bicelles, and lipidic cubic phase [21,22,24,29]. The crystals grew in clusters, resulting in overlap of diffraction patterns (Fig. 3), and thus it was not possible to assign a point group. However, we show that the crystals grown from TPA solubilized bR diffract to approximately 2.5 Å resolution. Optimization of crystallization conditions may yield larger crystals that either grow singly, or can be detached from their neighbors.

It has been shown previously that crystals of bR solubilized with *n*-octyl β-D-glucopyranoside could be grown as large as 600×300×100 μm which diffract to 2.9 Å resolution [25,26]. The 2.5 Å diffraction limit of the much smaller bR/TPA crystals could suggest a higher degree of internal order, perhaps due to TPA-mediated crystal contacts. However, better diffracting crystals can be obtained by incorporating bR solubilized in *n*-octyl β-D-glucopyranoside into the lipidic cubic phase using monoolein.

Tripod amphiphiles have not been extensively tested for solubilization of membrane proteins. Here, we have shown that, in addition to its previously reported utility for extracting and stabilizing bR, TPA also supports formation of well-diffracting crystals. The previously described novel detergents such as amphipols and lipopeptides can be useful for solubilization. However, because of relative sizes of these larger novel detergents, there can be problems in the formation of well-ordered contacts due to the potential disorder of the side chains of these novel detergents. Because of the size and rigidity of tripod amphiphiles, there is less interference by the detergent for the formation and disruption of protein contacts in the protein crystal. The results with TPA expand our knowledge of what structural motifs in detergents are useful for membrane proteins, and it is hoped that this work will engender interest in adding TPA to the array of commonly used detergents.

### Acknowledgements

We thank Kenton Longenecker, Vincent Stoll, and Clarissa Jakob for helpful comments. Diffraction data were collected at beamline 17-ID in the facilities of the Industrial Macromolecular Crystallography Association Collaborative Access Team (IMCA-CAT) at the Advanced Photon Source. The companies of the Industrial Macromolecular Crystallography Association support these facilities. Use of the IMCA-CAT beamline 17-ID (or 17-BM) at the Advanced Photon Source was supported by the companies of the Industrial Macromolecular Crystallography Association through a contract with Illinois Institute of Technology.

Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, and Office of Basic Energy Sciences, under Contract No. W-31-109-Eng-38. Work at the University of Wisconsin was supported by the Robert Draper Technology Innovation Fund.

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