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Structural analysis of bacteriorhodopsin solubilized by lipid-like phosphocholine biosurfactants with varying micelle concentrations

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ABSTRACT

Surfactants that can provide a more natural substitute for lipid bilayers are important in the purification and in vitro study of membrane proteins. Here we investigate the structural response of a model membrane protein, bacteriorhodopsin (BR), to phosphocholine biosurfactants. Phosphocholine biosurfactants are a type of biomimetic amphiphile that are similar to phospholipids, in which membrane proteins are commonly embedded. Multiple spectroscopic and zeta potential measurements are employed to characterize the conformational change, secondary and tertiary structure, oligomeric status, surface charge distribution and the structural stability of BR solubilized with phosphocholine biosurfactants of varying tail length. The process of phosphocholine micelle formation is found to facilitate the solubilization of BR, and for long-chain phosphocholines, concentrations much higher than their critical micelle concentrations achieve good solubilization. Phosphocholine biosurfactants are shown to be mild compared with the ionic surfactant SDS or CTAB, and tend to preserve membrane protein structure during solubilization, especially at low micelle concentrations, by virtue of their phospholipid-like zwitterionic head groups. The increase of alkyl chain length is shown to obviously enhance the capability of phosphocholine biosurfactants to stabilize BR. The underlying mechanism for the favorable actions of phosphocholine biosurfactant is also discussed.

1. Introduction

Membrane proteins are estimated to represent 20–30% of all genes in most genomes and perform a variety of functions vital to the survival of organisms [1–3]. These proteins are also the targets of the majority of clinical drugs [4]. The inherent insolubility of membrane proteins, however, has made it difficult to isolate and manipulate them compared with soluble proteins, and understanding of their structure and function lags far behind that of soluble proteins [http://blanco.biomol.uci.edu/Membrane_Proteins_xtal.html]. To avoid membrane protein aggregation or/and denaturation outside their native lipid bilayer environment, surfactants/detergents are indispensable in the isolation and purification of membrane proteins for subsequent structural determination and biophysical and biochemical characterization.

Based on the charges of the head groups, surfactants can be categorized into four types, anionic, cationic, zwitterionic and nonionic surfactant. The hydrophobic chain of a surfactant allows the molecule to partition into the apolar lipid bilayer during solubilization of membrane proteins. It also adsorbs to and masks the hydrophobic portions of these proteins once they have been solubilized and thus prevents protein aggregation [5]. So far the selection of an appropriate surfactant is one of the central technical difficulties in membrane protein study, especially for structure determination, since the variations in hydrophobic chain length, head group size or head group charges of surfactants are all closely related to their propensity to preserve or disrupt membrane protein structure [6–9]. Each surfactant can be characterized by its critical micelle concentration (CMC), the minimum concentration for surfactants to form micelles, which is another critical factor in surfactant applications [6–9]. In order to explore optimum conditions for membrane proteins, surfactant micelles have been employed as mimics of the native lipid bilayer environment to solubilize and stabilize membrane proteins. A surfactant micelle that is able to mimic the physicochemical properties of the original phospholipids is expected to be optimum at maintaining the structural integrity and stability of a given membrane protein.

The biomimetic phosphocholine surfactants are similar to phospholipids, in which membrane proteins are commonly embedded. They have phospholipid-like zwitterionic head groups, but their hydrophobic tails contain only a single alkyl chain, and they are water soluble either as monomers or as micelles. In contrast, phospholipids have low solubility as monomers and tend to aggregate

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into planar bilayers, which are water insoluble. Phosphocholines, however, also bear the same common structural features as conventional surfactants, and the presence of bioactive functionality in their head group introduces new features such as biomembrane-like surface charge distribution and biological specificity for phosphocholine micelles. Thus, the phosphocholine surfactants that bridge the structural or functional differences between phospholipids and conventional surfactants [e.g., *n*-dodecyl-*β*-D-maltopyranoside (DDM) and *n*-octyl-*β*-D-glucopyranoside (OG)] may be an ideal substitute for biological phospholipids in membrane protein extraction and solubilization. Indeed, phosphocholine surfactants have been shown to be highly efficient in solubilizing several types of membrane proteins, such as G protein-coupled receptors (GPCRs), water channels and membrane-bound enzymes [10–12]. In particular, it has been observed that these surfactants repeatedly show better performance in extracting different GPCR subtypes expressed heterologously in mammalian or bacterial cells than conventional surfactants or their mixtures [13–18]. Despite their potential usefulness as solubilizing agents for a broad spectrum of membrane proteins, it is still not known to what extent phosphocholine surfactants will interfere with the proper folding of proteins and the crystallization of GPCRs as well as other types of membrane proteins. In this work, the evaluation of how membrane protein structures respond to phosphocholine solubilization has been carried out with bacteriorhodopsin (BR) as the model protein.

BR is a bacterial membrane protein of known structure and homologous to GPCRs of higher organisms and is composed of seven transmembrane alpha-helices with one retinal moiety enclosed inside. This protein acts as a light-driven proton pump, converting light energy into a proton gradient, and is the only protein constituent of the purple membrane (PM), a two-dimensional crystal lattice naturally present as part of the plasmic membrane of *Halobacterium salinarum* [19]. In addition to the presence of BR, about 25% of the PM are lipids, of which 10% are non-polar lipid squalenes, 30% are glycolipids and 60% are phospholipids, with a ratio of about 10 lipids per BR [20]. BR by itself is extremely hydrophobic and is soluble in aqueous solution only in the presence of its native lipids or other external solubilizing agents. The retinal moiety linked via a protonated Schiff base to residue Lys216 of BR is a sensitive reporter of any small changes in its environment. By using UV–Vis absorption spectroscopy to monitor the change of the spectrum of the retinal group, the solubilization and denaturation process for BR can be followed with high precision [21,22].

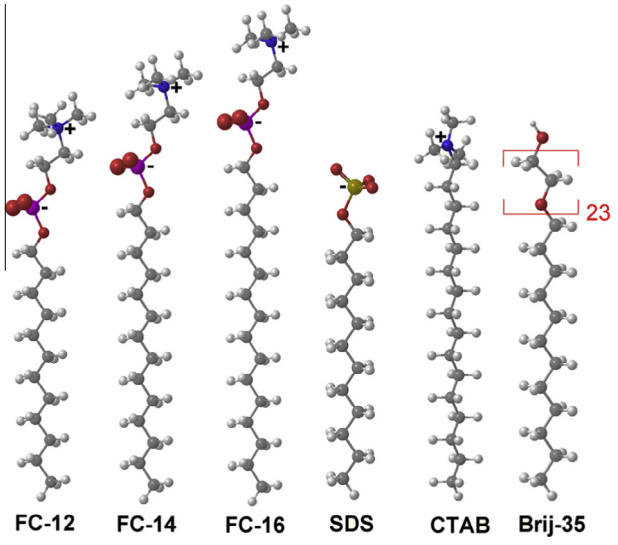

Fig. 1. Chemical structures of phosphocholine biosurfactants and other surfactants used in this work. The charge distribution of the head groups are shown for neutral pH conditions. Only one repeating unit of the head group of Brij-35 is drawn.

The mechanistic details that are relevant to membrane protein solubilization with surfactants have not yet been fully resolved. It is desirable to gain a more comprehensive understanding of the process and to optimize the use of surfactants for solubilization and maintenance of the structure of membrane proteins. Here, we have investigated the structural stability of BR when solubilized by phosphocholine surfactants. Since surfactant micellization is relevant to the solubility and stability of membrane proteins [6–9,23], phosphocholine surfactants are evaluated in a broad range of concentration from below to well above their specific CMC. Three phosphocholines, n-dodecylphosphocholine (FC-12), n-tetradecylphosphocholine (FC-14) and n-hexadecylphosphocholine (FC-16) are compared. Three other types of surfactants are also used for comparison, anionic sodium dodecyl sulfate (SDS), cationic hexadecyl (cetyl) trimethylammonium bromide (CTAB) and nonionic polyethylene glycol (23) monododecyl ether (Brij-35). The chemical structures of these surfactants are shown in Fig. 1. UV–Vis absorption, circular dichroism (CD) and fluorescence spectroscopic measurements combined with zeta potential analysis are employed to follow the responses of the BR structure to solubilization by these surfactants.

2. Materials and methods

2.1. Sample preparation

The PM fragments from *H. salinarum* were purchased from Sigma–Aldrich (product no. B0184). Before treated with surfactant, PM suspension in PBS buffer (10 mM, pH 6.2) was irradiated with light first to ensure the formation of light-adapted BR [24]. All the surfactants used were purchased from Affymetrix including FC-12 (CAS no. 29557-51-5), FC-14 (CAS no. 77733-28-9), FC-16 (CAS no. 58066-85-6), SDS (CAS no. 151-21-3), CTAB (CAS no. 57-09-0) and Brij-35 (CAS no. 9002-92-0). The chemical structures of these surfactants are as shown in Fig. 1. The surfactants were each mixed with the PM suspensions at room temperature for 10 h to extract BR from the PM fragments. The total BR concentration in PM was controlled at 2 μM, as determined spectroscopically by the extinction coefficient of 62,700 M^{-1} cm^{-1} at 568 nm [24]. The concentrations of the surfactants tested were controlled based on their specific CMC, from 0.3× to 200× CMC. According to the measurements by the supplier (www.anatrace.com), the CMC values of FC-12, FC-14, FC-16 and Brij-35 in H_2O are around 1.5 mM, 0.12 mM, 0.013 mM and 0.091 mM, respectively. The CMC values of SDS and CTAB are reported to be around 8.2 mM and 0.92 mM, respectively [25]. By surface tension method, similar CMC values were obtained with the same PBS buffer as used in our experiments (data not shown). Thus the concentrations of the surfactants tested were set according to the above CMC values. The solubilized BR samples were separated from the insoluble part as necessary by centrifugation at 16,100*g* for 30 min in a Sigma mini-centrifuge.

2.2. UV–Vis absorption spectroscopy

UV–Vis absorption spectra were recorded at room temperature on a UV-2450 UV–Vis spectrophotometer (Shimadzu). A cuvette with an optical path length of 1 cm was used in the measurements. For each surfactant, varying micelle concentrations were applied to the solubilization of PM. After incubation for 10 h at room temperature, the mixtures of a surfactant and PM with a protein concentration of 2 μM were centrifuged at 16,100*g* for 30 min. The supernatant containing the solubilized BR was removed, and the pellet was resuspended in an equivalent volume of PBS buffer.