SANS with contrast variation study of the bacteriorhodopsin-octyl glucoside complex

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Abstract. Membrane proteins (MPs), which play vital roles in trans-membrane trafficking and signalling between cells and their external environment, comprise a major fraction of the expressed proteomes of many organisms. MP production for biophysical characterization requires detergents for extracting MPs from their native membrane and to solubilize the MP in solution for purification and study. In a proper detergent solution, the detergent-associated MPs retain their native fold and oligomerization state, key requirements for biophysical characterization and crystallization. SANS with contrast variation was performed to characterize BR in complex with OG to better understand the MP-detergent complex. Contrast variation makes it possible to not only probe the conformation of the entire structure but also investigate the conformation of the polypeptide chain within the BR-OG complex. The BR-OG SANS contrast variation series is not consistent with a compact structure, such as a trimeric BR complex surrounded by a belt of detergent. The data strongly suggest that the protein is partially unfolded through its association with the detergent micelles.

1. Introduction
Membrane proteins (MPs) perform many vital cellular functions including membrane transport and trans-membrane signaling and make up roughly 30% of the expressed proteome. MPs are also the targets for many pharmaceuticals. The study of MPs continues to pose considerable technical challenges that result from their insolubility due to their hydrophobic character and the complex character of their natural environment. As a specific example, the number of unique atomic-resolution MP structures in the PDB, being less than 200 as of April, 2009 [1, 2], is dwarfed by the number of unique structures of soluble proteins. In spite of the challenges, the study of MPs continues to be driven by their biological importance.

Detergents are employed to both extract the MP from the native membrane and to maintain it in solution for further study. The traditionally assumed picture of a detergent-solubilized MP is that of a properly-folded MP in its native oligomerization state with the membrane-spanning, hydrophobic segments encapsulated in a belt of detergent [3], which is supported by crystal structures of membrane proteins showing detergent molecules clearly associated with membrane-spanning domains. For instance, the crystal structure of the OmpF porin from *E. coli*, a beta-barrel MP, has a single molecule thick ring of ordered detergent molecules encompassing the transmembrane surface of the structure

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High-resolution structures of alpha-helical MPs, such as bacteriorhodopsin (BR) crystallized with detergent [5] and lipids [6] also support the existence of a detergent belt.

A recent SAXS study of BR in complex with octyl glucoside (OG), the detergent used to obtain the atomic-resolution structure [5], found that the protein contained significant alpha-helical secondary structure while displaying SAXS data consistent with unfolded structure [7]. The data demonstrated that the traditional model of a detergent-solubilized MP in solution is not always accurate, a problematic possibility for functional studies of MP-detergent complexes. To better understand the nature of this structure, small-angle neutron scattering (SANS) with contrast variation was used to probe the BR-OG complex. The results demonstrate that the structure of the BR in the complex is not consistent with a properly folded complex consistent with the expected, native trimeric structure.

2. Materials and Methods

2.1. SANS Data Collection

The BR-OG complex was prepared as previously described [7]. BR was dissolved (final 5 mg/mL) in a buffer containing 1% OG, 20mM PBS (pH =7.6), NaCl 100mM, and 0.02% NaN3, at different H2O/D2O ratios. SANS data were collected using the CG-2 General Purpose SANS instrument at the High Flux Isotope Reactor of Oak Ridge National Laboratory under cooperative agreement with the Center for Structural Molecular Biology, operator of the Bio-SANS instrument [8]. Two sample-to-detector distances of 2.36 m and 8.36 m were employed. The wavelength, λ, was set to 4.75 Å, with a wavelength spread, Δλ/λ, of 0.15. Data reduction followed standard procedures to correct for detector sensitivity, dark current and buffer solution background. Data were azimuthally averaged to produce I(q) vs. q, where q = (4πsinθ)/λ, where 2θ is the scattering angle from the incident beam. Data from the two detector distances were merged using software developed at the National Institutes of Standards and Technology [9].

2.2. SANS Data Analysis

Data were analyzed according to Debye [10] for the radius of gyration, Rg. The “beads-on-a-string” model for the structure factor, S(q) [11, 12], was also applied to the data. This model accounts for correlations between flexibly linked particles, such as micelles along the length of an unfolded protein, and provides the fractal dimension of the structure, D, and the correlation length between particles, ξ. A form factor, F(q), of a core-shell ellipsoid of revolution [13] was used to model the detergent micelles using scattering length densities calculated from the chemical structure of OG. The micelle structural parameters (ellipsoidal semi axes and shell thickness), D and ξ were free parameters in the data fitting. The OG micelle structural parameters were allowed to range around previously determined values [14].

3. Results

The SANS contrast variation series of data collected for the BR-OG system is shown in Figure 1. The data do not indicate that the BR-OG complex is a compact particle. The data collected in high D2O content solutions, where both the protein and detergent contribute to the scattering, are linear at low q on the log-log plot, suggestive of a fractal character. The data set collected at 30% D2O shows very little signal, indicating that the scattering length density of the solution is very near to the average of the BR-OG complex.

The match point of OG, as calculated from the chemical structure of the molecule, is 18.7%. Therefore, the SANS data collected in 15% D2O is dominated by the scattering from the BR. The Rg determined from the data set using the Debye model [10], is 152 ± 11Å when fit to a maximum q of 0.05 Å⁻¹ to avoid the noise at higher q-values, which is a much larger structure than is expected for the native trimeric form of BR [5] embedded in a belt of detergent. The forward scatter, I(0), of the 15% D2O data indicates that the structure consists of ~7-8 BR polypeptide chains, presumably in an extended structure that links multiple OG micelles. The results of fitting the BR-OG data collected in
94% D$_2$O are shown in Figure 2. The data set is fit very well by the “beads-on-a-string” of the model [11,12]. The OG micelle determined from the fitting is an oblate core-shell ellipsoid 75 Å in diameter and 50 Å thick. The fractal dimension of the $S(q)$ is 1.23 ± 0.02, consistent with an open structure [11,12] and $\zeta$ is 30.1 ± 1.0 Å.

**Figure 1.** SANS contrast variation series data collected for the BR-OG complex. The curves have been offset for clarity.

**Figure 2.** (top) Fit of the BR-OG SANS data in 15% D$_2$O for $R_g$ according to Debye [10]. (bottom) Fit of the 94% D$_2$O data using the “beads-on-a-string” model [11,12].

### 4. Discussion

The SANS contrast variation series collected of the BR-OG complex demonstrates that the complex is not a compact structure. Instead, the BR within the complex appears to be both partially unfolded and in a non-native oligomerization state. The data collected at high scattering length density contrast suggest that the BR-OG complex forms an interconnected network. The data collected near the OG contrast match point clearly indicate that the protein is both not in a compact conformation and that the oligomerization state of BR is not the expected trimer [5].

The “beads-on-a-string” model agrees very well with the data, yet $\zeta$ is quite low. There are only ~150 OG monomers per BR polypeptide chain in the solution, which is very near the number of bound OG monomers per BR polypeptide chain found in a previous SANS study of the complex (~121 OG per BR) [15]. In a study of soluble proteins in complex with sodium dodecyl sulfate, $\zeta$ decreased significantly as the detergent-to-protein ratio decreased [16]. The critical micelle concentration of OG (~0.6% w/v) is also very near the concentration used (1% w/v), making it possible that not all of the OG are associated with the BR. The size of the micelle found by the “beads-on-a-string” fitting suggests that the OG has aggregated into larger structures interconnected by several protein chains.

The result is consistent with a previous SAXS study of the BR-OG complex by this group [7], but does not agree with a recent study of the complex by SANS [15]. The primary difference between the two experiments performed by the two groups is the use of BR from a commercial source in the present study versus the expressed and freshly purified BR studied by Santonicola and coworkers. Santonicola and coworkers concluded that the BR-OG complex was relatively compact. Close inspection of the published data [15] suggests that the scattering objects are not strictly monodisperse, or may not be compact. In particular, the BR-OG SANS curve presented in the upper panel of Figure 6 of reference [15] does not strictly have a slope of zero at low $q$, as it should for monodisperse, compact particles. Further, the Guinier plot shown in Figure 4 of reference [15] is not strictly linear.
The effect is subtle, and the $I(0)$ determined from the data supports a predominantly monodisperse sample. Other groups [17, 18] have also concluded that MP-detergent complexes are compact based on scattering data that appear to suggest an unfolded state similar to that observed in the present study. In contrast, the expressed and purified Ste2p G-protein coupled receptor was found to adopt the same partially-unfolded state as BR study while still showing significant alpha-helical secondary structure content [7]. The photosystem I complex purified from spinach was found to be at least partially unfolded while still retaining some level of functionality, having a SAXS profile that was not consistent with the crystal structure of the multisubunit complex embedded in a belt of detergent [19].

5. Conclusions
The present work demonstrates that commercially-obtained BR resolubilized by OG does not result in a correctly folded structure or the native oligomerization state, in contrast to other results that suggest that purified BR adopts a compact conformation in OG. Such a state would undoubtedly have consequences for the interpretation of functional studies or other biophysical characterizations of commercially-obtained BR. SANS can play an important role in the study of membrane proteins by making it possible to determine the true solution conformation and oligomerization state of a detergent solubilized membrane protein prior to investigation by other experimental methods.

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