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CHARACTERIZATION OF PHOTOSYNTHETIC SUPRAMOLECULAR ASSEMBLIES USING SMALL ANGLE NEUTRON SCATTERING.

D.M. Tiede¹, P. Marone¹, A.M. Wagner¹ and P. Thiyagarajan²

¹Chemistry Division D-200 and ²Intense Pulsed Neutron Source
Argonne National Laboratory, Argonne, IL 60439 USA

1. Introduction

We are using small angle neutron scattering (SANS) to resolve structural features of supramolecular assemblies of photosynthetic proteins in liquid and frozen solutions. SANS resolves the size, shape, and structural homogeneity of macromolecular assemblies in samples identical to those used for spectroscopic assays of photosynthetic function. Likely molecular structures of the supramolecular assemblies can be identified by comparing experimental scattering data with scattering profiles calculated for model supramolecular assemblies built from crystal structures of the individual proteins. SANS studies of the *Rhodobacter sphaeroides* reaction center, RC, presented here, show that the detergent solubilized RC exists in a variety of monomeric and aggregation states. The distribution between monomer and aggregate was found to depend strongly upon detergent, temperature and nature of additives, such as ethylene glycol used for low temperature spectroscopy and polyethylene glycol used for crystallization. Likely aggregate structures are being identified by fitting the experimental scattering profiles with those calculated for model aggregates built-up using the RC crystal structure. This work establishes the foundation for using SANS to identify intermediates in the RC crystallization pathways, and for determining likely structures of complexes formed between the RC and its physiological reaction partners, cytochrome c, and the LHI antenna complex.

2. Procedures

Deuterated RCs were isolated from *Rb. sphaeroides* R-26 cells grown on >98% ²H-enriched medium, using procedures described previously (1). RCs were placed in either 0.06% (w/v) lauryldimethylamine-N-oxide, LDAO, or 0.8% n-octyl-β-D-glucoside, OG, by washing a RC loaded DEAE Sephadex column with either buffer, and eluting with the same buffer containing 0.28 M NaCl. The ²H₂O/¹H₂O ratios were adjusted by dialysis to be 5% and 17% for the LDAO and OG samples respectively. These ratios were chosen to "contrast match" the neutron scattering length densities of the detergents to that of the solvent, thus effectively eliminating the detergent contribution to SANS. Deuterated RCs were used to maximize their scattering amplitude. SANS data were collected at the Intense Pulsed Neutron Source of Argonne National Laboratory, using the small-angle

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diffractometer. Scattering profiles were calculated from crystal structures using a previously described algorithm (2).

3. Results and Discussion

3.1 Effect of Detergents

SANS data show that RCs in LDAO are monodispersed and unaffected by ionic strength. This can be illustrated by standard Guinier plots (3), in which the logarithm of the measured scattered neutron intensity, $\ln[I(q)]$, is plotted versus q^2 , where $q=4\pi\sin(\theta/\lambda)$, θ is the half scattering angle, and λ is the neutron wavelength. The square root of the slope will be proportional to the radius of gyration, R_g , for homogenous, non-interacting particles of any type in the region where $qR_g < 1$, and it will be proportional to the z-averaged R_g for polydisperse systems. Figure 1 shows Guinier plots for LDAO solubilized RCs in low (10 mM tris) and high (10 mM tris, 1 M NaCl) ionic strength. Both plots are well fit by single slopes corresponding to R_g values of 31 ± 2 Å and 29 ± 1 Å respectively. The consistency of the slopes shows that the RC particle size is uniform and essentially independent of ionic strength.

Significantly different behavior is seen for RCs solubilized in OG solutions, as illustrated by the Guinier plots in figure 2, shown for samples in both 10 mM Tris and 10 mM Tris, 1 M NaCl. Both show increases in slopes at low q -values, reflecting the presence of RC aggregates and size dispersity in these samples. In addition, the slopes of Guinier plots for samples at low ionic strength are steeper than those at high ionic strength. The fitted regions in the Guinier plots in figure 2 correspond to R_g values of 40 ± 1 Å and 32 ± 1 Å for the low and high ionic strength samples respectively. These results suggest that the RC particle size distribution in OG solutions is shifted by high ionic strength to smaller sizes, and approach those seen in LDAO solutions.

Likely structures for RC particles in detergent solubilized solutions can be identified by fitting experimental SANS data with scattering profiles calculated from the RC crystal coordinates. Figure 3 shows the comparison between the scattering profile calculated for RC monomers based upon the crystal structure (4) and the experimental data measured for LDAO solubilized RCs at high ionic strength. The close agreement between both curves shows i) that LDAO solubilized RCs are predominately monomeric with a structure consistent with the crystal coordinates, and ii) that the contrast matching technique has successfully removed scattering from the detergent, allowing the scattering curves to be analyzed in terms of the deuterated RC components exclusively. This latter fact establishes the validity of using SANS measurements on deuterated photosynthetic proteins under detergent contrast matching conditions to study structures of supramolecular assemblies without interference from detergent components.

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Preliminary modeling corroborates the finding that OG solubilized RCs are a mixture of monomeric and aggregated forms. The distribution shifts toward a monomer at high ionic strength. The aggregates appear to be approximately linear, and span the size range from dimers to at least octamers.

Figure 1 Guinier plots for RCs in 0.06% (w/v) LDAO, 10 mM Tris pH 7.8, ●, and the same buffer with 1 M NaCl, ○.

Figure 2 Guinier plots for RCs in 0.8% (w/v) OG, 10 mM Tris pH 7.8, ●, and the same buffer with 1 M NaCl, ○.

Figure 3 Experimental SANS profile for RCs in 0.06% LDAO, ●, and calculated profile from RC crystal coordinates, --.

Figure 4 Guinier plots for RC in 0.8% OG, 10 mM Tris, pH 7.8, 22°C, ●, and 6°C, ○.

3.2 Effect of Temperature

Temperature in the range 22°C to 6°C was not found to affect the SANS of LDAO solubilized RCs, reflecting the independence of RC particle size on temperature in this range. In contrast, significant temperature-dependent changes were seen for SANS profiles of OG solubilized RCs. Figure 4 shows Guinier plots of SANS data for an OG solubilized RC sample measured at 22°C and 6°C. The data collected at 6°C shows increased

scattering intensity at low q values. The slopes for equivalent portions of Guinier plots yield R_g values of 53 ± 1 Å and 39 ± 1 Å for the 6°C and 22°C data respectively. These results show that an appreciable shift toward larger RC particle sizes occurs upon cooling OG solubilized RCs that was not seen in LDAO solutions.

For both LDAO and OG solubilized RCs extensive three-dimensional aggregation occurs upon solution freezing. A scattering profile for LDAO solubilized RCs at -250°C is shown in figure 5. The significant increase in scattering at low q values reflects the increase in particle size. Aggregation is expected to occur due to phase separation during freezing of aqueous samples. However, the scattering profiles for frozen solutions also show a dramatic decrease in scattered intensity in the q -range 0.01 to 0.1 Å $^{-1}$. Scattered intensity in this region arises from the RC shape. The SANS data for frozen solutions show that all information about RC shape is lost. This result can only occur in the present experiments if the interface is lost between the RC and the contrast matched water and detergent phases. Such conditions imply the existence of aggregation in which there are extensive, direct RC-RC contacts. Recovery from this state upon thawing is found to take one to several hours.

3.3 Effect of Co-Solutes

Solutions of 50% ethylene glycol or 50% glycerol are typically used for spectroscopic characterization of RCs at low temperature. For both LDAO and OG solubilized RCs, these co-solutes were found to cause an aggregation of RCs at 22°C that could not be altered by changes in ionic strength nor increases in detergent concentration. Figure 5 shows the scattering profile for LDAO solubilized RCs in 50% ethylene glycol at -250°C . Only slight changes in scattering profiles were seen upon cooling. Preliminary modeling suggests that under the conditions used for low temperature spectroscopy RCs exist predominately as approximately linear aggregates.

Figure 5 SANS for RCs in 0.06% LDAO at 22°C , $\bullet$, -250°C , $\circ$, and in 50% ethylene glycol - 250°C , Δ .

Polyethylene glycol, PEG, which is used for the crystallization of RCs, was also found to cause RC aggregation at concentrations below the crystallization/precipitation threshold. The similarity in the induction of RC aggregation by PEG, ethylene glycol and glycerol suggests that the effect is not reagent specific, but is driven by a generalized osmotic effect. The extent of RC aggregation was found to increase dramatically as the PEG concentration approaches the crystallization threshold of 13.6% (w/v) in 0.28 M NaCl. The results indicate that < 20% of RCs are likely to be in the monomeric form in crystallization mixtures. Experiments that remove RC aggregates from crystallization mixtures suggest that the disaggregation of RCs is likely to be a critical, rate-determining step in RC crystallization, and that this is the event that needs to be optimized for successful crystallization.

4. Conclusions

The SANS data presented here characterize the aggregation state of the bacterial RC under conditions typically used in assays of photosynthetic function. The results show that in aqueous buffers, RCs solubilized by LDAO are more monodispersed than with OG, and that OG solubilized RCs also show a surprising temperature dependent aggregation. The use of glass forming aqueous mixtures, solution freezing, and PEG additions used for crystallization caused prominent aggregation of RCs in both detergents. This work establishes protocols for the investigation of the structures of supramolecular formed between the RC and its physiological reaction partners.

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