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In situ SAXS measurements of structural modifications of lipidic mesophases in bacteriorhodopsin crystallization *in meso*

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1 Introduction

In meso membrane protein crystallization approach, where the lipid cubic or sponge phase is used as a crystallization matrix has recently been found applicable to various membrane proteins, e.g., GPCR's, which for a long time failed to be crystallized by other crystallization approaches. However, the roles of the mesophase structures in the crystallization process remains far from being understood. Taking a bacteriorhodopsin (bR)/β-XylOC_{16+4} crystallization system [1] as an example, we here report an attempt to monitor changes in the mesophase structures occurring in the bR crystallization process *in situ*.

2 Experiment

We have employed a sandwich crystallization cell (5 mm ϕ, 135 μm in thickness) shield by a pair of 50 μm thick glass windows, where a 200nl of bR/β-XylOC_{16+4} mixture and 1 μl of crystallizing solutions (1.5, 2.3, 3.0 M Na_2HPO_4/KH_2PO_4 pH5.6) were delivered in each well. The SAXS measurements were performed at BL-4A at 20 ± 0.5 °C. Exposure time was 5 seconds at a sample to film distance of ~70 cm.

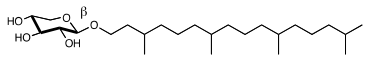

Fig. 1. Chemical structure of a matrix lipid, 1-O-(3,7,11,15-tetramethylhexadecyl)-β-D-xyloside, (β-XylOC_{16+4})

3 Results and Discussion

Upon addition of the crystallizing solution, the homogeneous bR/β-XylOC_{16+4} mixture was rapidly separated into a bR-poor peripheral region and a bR-rich central region, which eventually resulted in a crystal formation (Fig. 2).

The SAXS measurements indicated that the morphological changes are accompanied by changes in the mesophase structures (Fig. 3). Initially, the bR/β-XylOC_{16+4} mixture was a lamellar phase, Lα. Upon addition of the crystallizing solution, a sponge phase, L3, started to form at the expense of the Lα phase, which disappeared after 2-4 hrs. The L3 phase then gradually transformed into a *Pn3m* cubic phase, whose lattice constant continuously decreased with time, e.g., after 1 week, it reached about 17.5, 16, and 12 nm for 1.5M, 2.3M, and 3M of Na_2HPO_4/KH_2PO_4, respectively (Fig. 3).

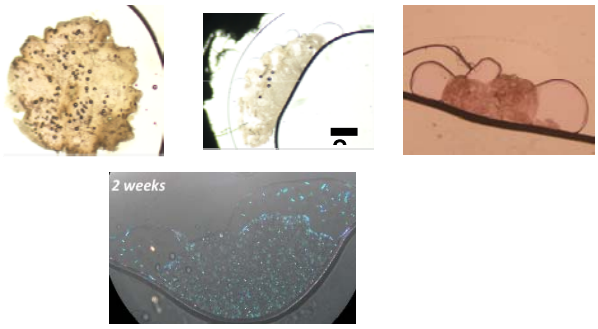

Fig. 2. Morphological changes in the bR crystallization *in meso* {1.5M Na_2HPO_4/KH_2PO_4 pH5.6} (5 min, 3h, 4 days from left to right, and 2 weeks,

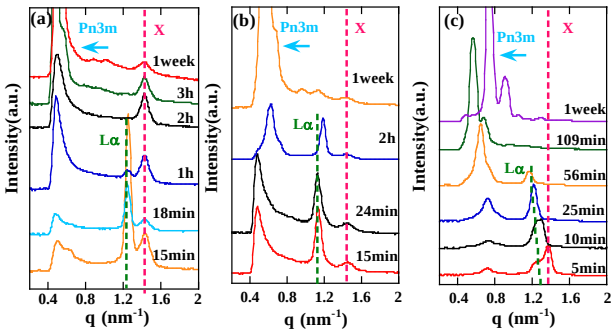

Fig. 3. Time dependent SAXS profiles observed during crystallization of bR for three different crystallization conditions. 1.5 M (a), 2.3M (b), and 3.0M (c)Na_2HPO_4/KH_2PO_4 pH5.6, respectively.

Summary

We have developed a convenient method for *in situ* SAXS measurements of the *in meso* membrane protein crystallization processes.

References

[1] V. Borshchevskiy, et al., *J. Cryst. Growth*, **312**, 3326 (2010).

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