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Structure of mixed micelles composed of phospholipid and detergent

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Introduction

Bicelle is a bilayered disc-shaped mixed micelle composed of phospholipids and detergent, and has unique advantages as a model biomembrane system that provides hydrophobic circumstances for the membrane proteins. Nm sized bicelles can be a good candidate for the medium of structural investigations of membrane proteins. DMPC and CHAPSO form bicelles in aqueous media, and exhibits various assembled structure corresponding to the solution conditions. In order to characterize the assembled structure of bicelle in detail and to obtain the fundamental information for the utilization of bicelle in the structural analyses of membrane proteins, small angle X-ray scattering and light scattering investigations were carried out.

Experimental

Characterization of mixed micelles at various solution conditions were carried out by means of BL-10C combined with LS measurements. Details of the samples and the preparations are described elsewhere [1].

Results and Discussion

Mixtures of DMPC and CHAPSO form mixed micelles at various conditions, and it was observed that the shape of them are rotational ellipsoid-disc-rod-vesicle depending on the composition ratio q (= [DMPC]/ [CHAPSO]), the total concentration C_t (= [DMPC]+ [CHAPSO]), and the temperature. For example, at the condition of C_t = 30 mM and 30°C, the mixture forms disc-shaped bicellar molecular assembly with very narrow size distribution in the q range of ca 0.5 to ca 1.0. With increasing q, the shape of assembly becomes rodlike and vesicle. By means of light scattering and SAXS, it was ascertained that the thickness of disc-shaped assembly is almost constant and equals with that of the lipid bilayer. The scattering functions of SAXS showed very characteristic behavior suggesting the regular spatial arrangement of electron density, and this point is exhibited in the distance distribution function, too. The scattering functions are well reproduced by the model structure introducing the bilayered arrangement of phospholipid with CHAPSO molecules at the rim of the disc. Since the disc thickness evaluated by the model fitting for the mixture of DOPC, DLPC, DMPC, and DPPC with CHAPSO increases linearly with the increase of lipid chain length, the validity of such model structure is well established. Such a spatial profile suggests that the bilayer assembly of DMPC is edge-stabilized by CHAPSO molecules at the rim aligned in the direction of disc normal, and CHAPSO molecules are located also in the bilayered surfaces. The shape and size of assemblies change with C_t even for the constant q. These results are due to the molecularly dispersed CHAPSO molecules coexisting in the solution, and the composition enrolled in the mixed micelle effectively varies with C_t. In order to estimate the molecularly dispersed CHAPSO concentration, mapping of size and shape of assemblies over the wide concentration range of DMPC and CHAPSO, and it was about 2 ~ 2.5 mM. Based on this finding, the molecular weights M_w of bicellar assemblies were obtained by the conventional static light scattering measurements.

With increasing q, M_w increases gradually in the q range of 0.5 to 1.0 (the region of bicelle formation) and increases rapidly at the q range larger than 1.0 (rod formation). Postulating that uniform disc-shaped assemblies are formed, the respective quantities of DMPC and CHAPSO molecules embedded in the disc were calculated. For example, those were 93 (DMPC) and 84 (CHAPSO) for the mixture of q = 1.0, C_t = 50 mM at 30°C. Combining with the disc radius obtained by SAXS, and assuming that the surface area per one DMPC molecule in the bilayered surface is 0.717 nm^2 and the width of one CHAPSO molecule at the rim is 1 nm, it was clarified that CHAPSO molecules locate substantially in the disc surface as well as at the rim. Surface area occupied by one CHAPSO molecule in the disc surface decreases with q (1.30, 0.76, and 0.32 nm^2 for q = 0.5, 0.75, and 1.0, respectively). This fact means that CHAPSO molecules is necessary to form bicellar (flat surface) structure, and is inserted in the disc surface with the sterol pseudoplane oriented parallel to the surface at low q (0.5) and normal to the surface at larger q (1.0). When the surface area available for CHAPSO molecules are too small, the disc-shaped assembly becomes destabilized and the assembly exhibits disc-to-rod shape transition (bicelle is unstable without CHAPSO). The fact that CHAPSO molecules align parallel to the disc surface at low q indicates that plane structure of CHAPSO molecule (steroid ring) is likely to be packed parallel to the micelle surface, and such a manner of accumulation is not easy resulting a low micellar aggregation number. The combination of q = 0.5, C_t = 50 mM at 30°C gives disc diameter being 5.3 nm and is sufficiently small for the high speed rotational movement to establish good NMR signal and corresponds to 0.69 mM of bicelle, then is convenient for embedding proteins (1 protein to 1 bicelle). When protein molecules are embedded into the bicelle, the aligning manner of CHAPSO molecules could be modified to make effective the coexistence of protein molecules (from parallel to normal manner), and the disc size could be expected not to change significantly

References

[1] K. Kubota et al., PF Activity Rep. **19**, 147 (2002).
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