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Effect of glycosphingolipid on amyloid transition of apomyoglobin

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Introduction

Recently many proteins have been found to be involved in diseases through misfolding, which are called protein-folding diseases such as amyloidosis. Amyloid structures of proteins are stable but different from those native structures that are encoded in the amino acid sequences. The proteins forming amyloid deposits both in extra and intra cells possess diverse features in structural classes, sequence patterns and intramolecular interactions. Thus, the formation of amyloid aggregates would not be special for some distinctive proteins but may be ruled by some general physicochemical mechanism. Several globular proteins such as apomyoglobin and lysozyme have been found to form amyloid fibrils. The studies of amyloid formation of these proteins under various conditions might possibly clarify an insight on the general mechanism. On one of the candidates effectively inducing amyloid formation, previous spectroscopic studies using fluorescence spectroscopy and circular dichroism [1, 2] showed that amyloid β proteins (Aβ) interact strongly with monosialogangliosides (G_{M1}) to promote a structural transition of Aβ from helix to sheet and that G_{M1}-bound Aβ is suggested to form seeds in the course of Aβ polymerization to amyloid fibril (Hayashi *et al.*, 2004). G_{M1} is one of the species of gangliosides. Gangliosides, major components of glycosphingolipids (GSLs), are rich in neuronal cells and form lipid microdomains, so-called rafts, with other particular lipids and proteins. Function of rafts is also one of the current hot topics in cell biology since rafts are assumed to have significant functions in signal transduction, cell adhesion and lipid/protein sorting. By using a model system of amyloid, we have carried out small-angle X-ray scattering (SAXS) to elucidate an initial stage of amyloid formation. Functions of membrane proteins evolved in signaling would be affected through accumulation of amyloid proteins.

Experimental

Apomyoglobin (ApoMY) from horse skeletal muscle, monosialoganglioside (G_{M1}) from bovine brain and cholesterol were purchased from SIGMA Chemical Co. (USA), which were used without further purification. All other chemicals used were of analytical grade. The small unilamellar vesicle containing [G_{M1}]/[cholesterol]=1/1 was prepared by the method as given elsewhere (Hirai *et al.*, 2003, 2005). The buffer solvents used were 50 mM Tris-HCl (2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride) buffer at pH 7-9. The final pH values of the samples were measured after the dissolution of proteins in buffers. The protein concentration for SAXS measurements were from 0.005 g/ml (2.8×10^{-4} mol/l) to 0.03 g/ml (1.7×10^{-3} mol/l). SAXS experiments were performed by using the spectrometer installed at BL-10C at PF. The X-ray wavelength, the sample-to-detector distance and the exposure time were 1.49 Å, 190 cm, and 300 s, respectively, where we used a one-dimensional position-sensitive proportional counter.

Results

Under the incubation condition at 328 K at pH 9, we have monitored the growing process of amyloid nuclei for ~8 hours. Fig. 1 shows the time dependence of radius of gyration after the addition of G_{M1} micelle and [G_{M1}]/[cholesterol]=1/1 mixed vesicle. The [G_{M1}]/[cholesterol]=1/1 mixture corresponds mostly to the raft fraction and forms a small vesicle (Hayakawa & Hirai, 2003). At the high molar ratio of [ApoMY]/[G_{M1}]=1/1 the growth of the ApoMY aggregate (amyloid ApoMY) is evidently suppressed for both cases of the additions of the micelle and the vesicle. Whereas, at the low molar ratio of [ApoMY]/[G_{M1}]=1/0.1 the growth of the aggregates is less suppressed and is as same as that of ApoMY without lipids. The present results would disagree with the previous report on the interaction of Ab with G_{M1} (Matsuzaki *et al.*, 1999). It might be occurred for different types of amyloid-like aggregates since ApoMY is much larger than Aβ. We need further experiments.

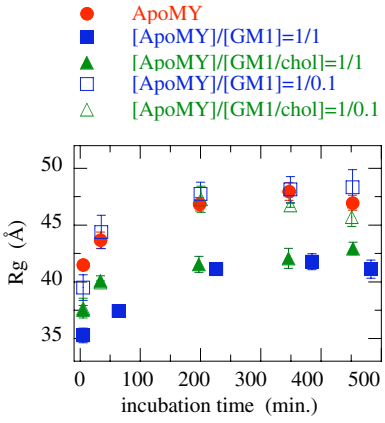

Fig.1 Incubation time dependence of the radii of gyration of ApoMY, ApoMY+G_{M1}, ApoMY+G_{M1}/cholesterol at 328 K at pH 9.0. The molar ratios of [ApoMY]/[G_{M1}] are indicated.

References

[1] L. P. C.-Smith, et al., Biol. Chem. **272**, 22987 (1997).
[2] K. Matsuzaki, & C. Horikiri. (1999). Biochemistry, **38**, 4137 (1999).