Phase behabior of glycoshpingolipid/cholesterol complex as a raft component

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[Introduction]

Recently, glycosphingolipids associated with cholesterol not only locate on outer-leaflet of cell membrane but also form lipid microdomains with other particular lipids and proteins in neuronal cells. The microdomains, so-called raft, are assumed to have a significant role as a molecular device to localize specific proteins which are evolved in cell-surface signal transduction such as src-family kinases and G proteins. Gangliosides, major components of glycosphingolipids, containing sialic acid residues, are abundant in the tissues of the central nervous system. Although the physiological functions of the microdomains are one of the current hot topics in cell biology, the biophysical studies of the formation and functionality of the microdomain are rather poor. Thus, we have carried out wide-angle X-ray scattering measurements to elucidate characteristics of ganglioside / cholesterol mixture depending on monovalent / divalent salt concentration as a model of the microdomains.

[Experimental]

Wide-angle scattering experiments were carried out by using a scattering spectrometer installed at BL-40B2. The sample-to-detector distance was 46 cm. The X-ray wavelengths used were 1.5 Å. Monosialoganglioside (G_{M1}) and cholesterol were purchased from SIGMA Chemical. Other reagents of special grade were used. The final concentration of ganglioside for all samples was 0.5 % w/v. The molar ratio of [ganglioside]/[cholesterol] was varied from 1/0 to 1/3. Monovalent and divalent salts were used.

[Results]

Figure 1 shows the structural changes of G_{M1}/cholesterol mixture depending on both [G_{M1}]/[chol] molar ratio and Ca^{2+} concentration. With increasing the molar ratio, a micelle-to-vesicle transition occurs in the vicinity of [G_{M1}]/[chol]= ~1/0.5, as indicating the change of the small-angle scattering profile below q= ~0.05 $Å^{-1}$ from a Gaussian-like shape to a steep one in Figure 1. For the micellar G_{M1}/cholesterol mixed aggregates, the addition of Ca^{2+} mostly does not affect the micellar structure up to [Ca^{2+}] = 50 mM. Whereas, the vesicular aggregate of G_{M1}/cholesterol mixture is greatly affected to show a vesicle-to-lamellar transition by the increase of Ca^{2+} concentration, as shown in Figure 1. The diffraction-peak position from inter-lamellar periodicity indicates that the bilayer-bilayer distance is 84.5 Å. According to the alkyl-chain and sugar-head lengths of G_{M1} molecule, the stacking of the bilayer in lamellar structure is supposed to be very tight, suggesting some inerdigitated structure formation of sugar-heads of G_{M1} molecules. Further results and discussion will be published elsewhere.

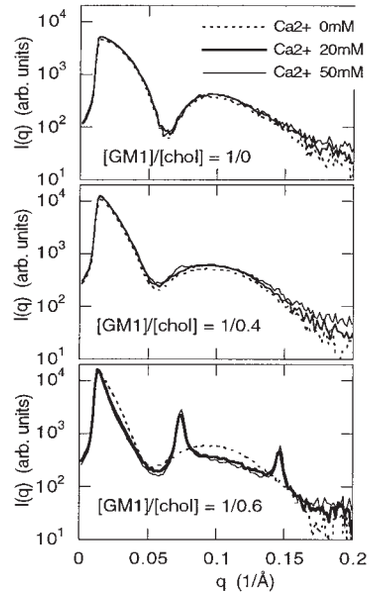

Figure 1. Structural changes of G_{M1}/cholesterol mixture depending on [G_{M1}]/[chol] ratio and Ca^{2+} concentration.

The Structural Study between the Heredity Diseases and the DNAs which have Noncanonical Structure

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The structure of left-handed DNA of the long chains is very much watched from doing relaxization of the super coil. It is because a part of the B-Z transition mechanism and the Z-B transition mechanism is explained if this structure becomes obvious. Relaxization mechanism of the super coil is made clear if a B-Z transition mechanism and a Z-B transition mechanism are made clear more. It is said that some suggestion is given to a cell proliferation and carcinogenesis mechanism about these facts. So, we cleared the stabilization mechanism of Z-DNA by the X-ray crystal structure analysis which a synchrotron radiation was used for, the molecule dynamics calculation, and so on. It thought whether the crystallization of Z-DNA of the long chain made unacceptable by to use how to stabilize that Z-DNA so far could be done, and we tried the crystallization of Z-DNA of the long chain. As we were thinking, it succeeded in the crystallization of Z-DNA of the long chain by using the stabilization technique of Z-DNA for the first time. When data measurement was done by using BL40B2 of SPring-8, this crystal reflected very well, and it could get the very good data of the 1.7 Å resolution.

A wavelength used in data measurement is 1.0 Å, and a camera length is 150mm, the rotation angle of phi is 1.5 degree by one frame. We can collected the data of 120 frame. The completeness of the data was 99% with R-merge of the data in 8.6% to 1.7 Å. Rotation and transrasion method were used, and Z-DNA of the long chain of the 1.5 molecule could be found in asymmetric unit. The refinement is being continued at present. The diffraction pattern of long Z-DNA was show in Fig. 1.

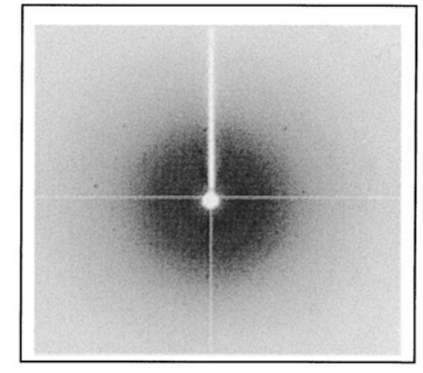

Fig.1 Diffraction pattern of long Z-DNA