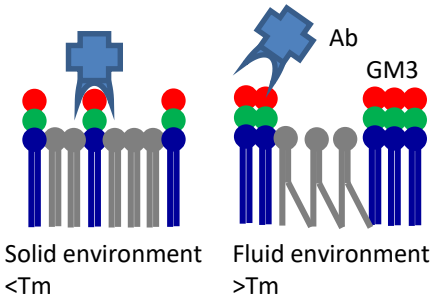
Principal Investigator : Toshihide Kobayashi

Grant Title : Distribution and function of glycolipids recognized by lipid-binding proteins

Abstract

GM3 (NeuAc-α2,3-Gal-β1,4-Glc-ceramide) is the simplest ganglioside and is the most common membrane-bound glycosphingolipid in tissues. GM3 is involved in a number of pathological conditions as diverse as cancer, hypercholesterolemia and diabetes (*1*). It is postulated that GM3 exerts the activity through GM3-rich lipid domains (*1*). Indeed the suppression of epidermal growth factor receptor autophophrylation by GM3 is shown to be lipid environment-dependent (*2*). On the plasma membrane, GM3 may form complex with other glycolipids, sphingomyelin or other GM3 molecules with different proportional ratio of cholesterol. However, little is known about the microenvironment surrounding GM3. Several monoclonal antibodies against GM3 have been established by Japanese scientists (*3-5*). Present study examined the binding characteristics of anti-GM3 antibody to GM3 in different membrane environment.

Anti-GM3 monoclonal IgM, GMR6, (hereafter Ab) showed environment-dependent binding to GM3. i.e. Ab bound GM3/dipalmitoylphosphatidylcholine (DPPC) mixture but not to GM3/dioleoylphosphatidylcholine (DOPC). The gel-to-liquid crystalline phase transition temperature (Tm) of DPPC is 41 °C whereas that of DOPC is -20 °C. Our results suggest that Ab binds GM3 only in rigid environment. Other Abs also showed environment-dependent binding. We then examined the temperature-dependence of the binding of Ab to GM3 mixed with different lipids. The binding of Ab to GM3/DPPC and GM3/palmitoyl sphingomyelin (SM) (Tm=40 °C) was dramatically decreased when the incubation temperature was higher than 37 °C. In contrast, the binding to GM3/glucosylceramide (GlcCer) (Tm=66-68 °C) was not affected until 42 °C. The binding to 100 % GM3 was not affected by incubation temperature. Comparison of FTIR spectra of GM3 by Muller et al (*6*) and our Raman spectra of SM (*7*) suggests that GM3 is monomer in solid phase and dimer or oligomer in fluid phase. Our results suggest that Ab failed to bind GM3 in fluid membrane due to the formation of GM3 dimer. It is speculated that at fixed lipid composition, the temperature dependence of the binding of Ab to GM3 is solely dependent on Tm. We then examined the temperature dependence of the binding of Ab to the cell surface. Different cells showed different temperature dependence, suggesting cell-dependent different Tm of GM3 microdomains. Temperature dependence was also altered in pathological conditions, suggesting the importance of microenvironment around GM3 in pathophysiology.



Model of Ab binding to GM3

References:

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