Cholesterol-dependent Formation of GM1 Ganglioside-bound Amyloid β-Protein, an Endogenous Seed for Alzheimer Amyloid*

Received for publication, January 11, 2001, and in revised form, March 12, 2001
Published, JBC Papers in Press, May 7, 2001, DOI 10.1074/jbc.M100252200

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GM1 ganglioside-bound amyloid β-protein (GM1/Ab), found in brains exhibiting early pathological changes of Alzheimer’s disease (AD) including diffuse plaques, has been suggested to be involved in the initiation of amyloid fibril formation in vivo by acting as a seed. To elucidate the molecular mechanism underlying GM1/Ab formation, the effects of lipid composition on the binding of Ab to GM1-containing lipid bilayers were examined in detail using fluorescent dye-labeled human Ab-(1–40). Increases in not only GM1 but also cholesterol contents in the lipid bilayers facilitated the generation of Ab to the membranes by altering the binding capacity but not the binding affinity. An increase in membrane-bound Ab concentration triggered its conformational transition from helix-rich to β-sheet-rich structures. Eximer formation of fluorescent dye-labeled GM1 suggested that Ab recognizes a GM1 “cluster” in membranes, the formation of which is facilitated by cholesterol. The results of the present study strongly suggested that increases in intramembrane cholesterol content, which are likely to occur during aging, appear to be a risk factor for amyloid fibril formation.

The critical step in the development of Alzheimer’s disease (AD)1 is the conversion of soluble, nontoxic amyloid β-protein (Ab) to aggregated, toxic Ab rich in β-sheet structures (1). Ab has been shown to form amyloid fibrils, but this requires concentrations of Ab (>10^−4 to 10^−3 M) (2–4) much higher than the physiological concentration (10^−6 M). Therefore, it has been hypothesized that aggregation of soluble Ab in vivo involves seeded polymerization (5, 6).

Yanagisawa et al. (7) discovered GM1 ganglioside-bound Ab (GM1/Ab) in brains exhibiting early pathological changes of AD and suggested that GM1/Ab may serve as a seed for toxic, amyloid fibril formation. Indeed, immunochromical (8, 9) and spectroscopic (10–13) studies demonstrated that GM1/Ab has a conformation different from that of soluble Ab and accelerates the rate of amyloid fibril formation of soluble Ab in vitro (12, 14). Interestingly, however, GM1/Ab is never found in the normal brain despite the fact that neuronal membranes are abundant in GM1 and physiological metabolism of amyloid precursor protein results in extracellular secretion of Ab. Thus, identification of factors that initiate formation of GM1/Ab may be crucial for determination of the pathogenesis of AD and for development of preventive and curative treatment strategies. We have recently found that alterations in lipid composition of the host membrane can be such a factor (13); generation of GM1/Ab is facilitated by the combination of cholesterol and sphingomyelin (SM) in membranes in proportions similar to the so-called detergent-insoluble glycolipid-rich domain (DIG) (15), suggesting that DIG is deeply involved in GM1/Ab formation. This hypothesis is in agreement with the observation that Ab is present in DIG in vivo (16, 17).

In this study, the effects of GM1 and cholesterol contents in the membranes on the binding of Ab to DIG-like lipid bilayers were examined in detail using fluorescent dye-labeled human Ab-(1–40). We report here that enrichment of cholesterol of the host membranes facilitated the generation of GM1/Ab via formation of a GM1 “cluster” that acts as a binding site of Ab. A plausible mechanism of onset of AD will be discussed.

EXPERIMENTAL PROCEDURES

Peptides—Human Ab-(1–40) labeled with the 7-diethylaminocoumarin-3-carbonyl group at its N terminus (DACA-Ab, Fig. 1) was custom synthesized by the Peptide Institute (Minou, Japan). The peptide was characterized by matrix-assisted laser desorption ionization mass spectroscopy (calculated, 4574.07; found, 4574.0) as well as amino acid analysis under different hydrolysis conditions. The dye-labeled peptide was always handled in light-protected, capped tubes under a nitrogen atmosphere to avoid photodegradation. Unlabeled human Ab-(1–40) was also purchased from the Peptide Institute. The latter peptide was first dissolved in 1,1,3,3,3-hexafluoro-2-propanol (Wako, Osaka, Japan) to avoid self-aggregation. After removal of the solvent by nitrogen purging, the peptide was redissolved in pure water (Nonapure) at 30 µM and then mixed with an equal volume of double concentrated buffer (20 mM Tris, 300 mM NaCl, 4 mM CaCl2, pH 7.4). The labeled peptide was found to be less stable in this organic solvent and therefore was directly dissolved in pure water. Physiological Aβ is present in a soluble form. To mimic this situation, we removed aggregates, if any, by ultracentrifugation in 500–1000 µl polyallomer tubes at 100,000 × g at 4 °C for 1 h. Indeed, the supernatant used adopted unordered structures (see

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‡ Supported by Grants-in-aid for Scientific Research on Priority Areas C, Advanced Brain Science Project, from the Ministry of Education, Science, Sports and Culture, Japan, and Research Grant for Longevity Sciences 11-C01 from the Ministry of Health and Welfare.

‡‡ Supported by Grants-in-aid for Scientific Research on Priority Areas B-12142020.

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1 The abbreviations used are: Ab, Alzheimer’s disease; Aβ, amyloid β-peptide; BODIPY-GM1, BODIPY® FL C5-GM1; DACA, 7-diethylaminocoumarin-3-carbonyl; DIG, detergent-insoluble glycolipid-rich domain; LUV, large unilamellar vesicle; PC, phosphatidylcholine; PS, phosphatidylinerine; SM, sphingomyelin; L/P, lipid/peptide ratio; GM1, monosialoganglioside GM1.
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Fluorescence intensity was denoted by \( I \), and the suffixes indicate polarization (in degrees) of the excitation-emission bands. Fluorescence intensity of the corresponding blank sample without BODIPY-GM1 was negligible.

CD—Native human Aβ(1–40) (15 μM) in buffer (10 mM Tris, 150 mM NaCl, 2 mM CaCl₂) was used for CD measurements. CD spectra were measured on a Jasco J-720 apparatus interfaced to an NEC PC8801 microcomputer, using a 1-mm path length quartz cell to minimize the absorbance due to buffer components. The instrumental outputs were calibrated with nonhygroscopic ammonium d-camphor-10-sulfonate (24). Eight scans were averaged for each sample. The averaged blank spectra (vesicle suspension or buffer) were subtracted.

RESULTS

Lipid Specificity of Aβ Binding—Binding of DAC-Aβ to LUVs of various lipid compositions was estimated on the basis of DAC fluorescence. The fluorophore was practically nonfluorescent in aqueous environments (Fig. 2A, trace 1). The addition of GM1-rich cholesterol-rich LUVs at a lipid/peptide ratio (L/P) of 80 induced a large increase in fluorescence intensity accompanied by a blue shift in the emission maximum from 483 to 470 nm, indicating that the peptide was bound to the membrane with the N-terminal DAC moiety embedded in a hydrophobic environment. Fluorescence spectra of DAC-Aβ were also measured in various dioxane/water mixtures. The maximal wavelength of 470 nm in the presence of the membrane corresponded to that in a dioxane/water (3/1, v/v) mixture with a dielectric constant of ~20, suggesting that the DAC moiety was located at the interfacial region of the membrane (25).

To confirm that DAC-Aβ behaves similarly to native Aβ, competitive binding experiments were carried out. The binding sites of GM1-rich cholesterol-rich LUVs were almost saturated with DAC-Aβ. Unlabeled Aβ was then added, and a decrease in fluorescence was monitored as a function of unlabeled Aβ-to-DAC-Aβ ratio (Fig. 2B, closed circles). About 40-fold unlabeled peptide was needed to replace 50% of the labeled peptide. Conversely, the pretreatment of the vesicles with excess unlabeled peptide decreased DAC-Aβ binding (Fig. 2B, open circle). These data suggest that both peptides competitively and reversibly bind to the common binding site with DAC-Aβ having a 40-fold larger affinity.

Thus, DAC fluorescence can be utilized to assess the binding affinity of Aβ for various membranes. As a quantitative measure, relative fluorescence enhancement (\( R \)) is defined as follows.

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R = \frac{F - F_0}{F_0} \quad (\text{Eq. 3})
\]

Fluorescence intensities at 466 nm (peak of raw, uncorrected spectra) in the presence and absence of LUVs are denoted by \( F \) and \( F_0 \), respectively. Fig. 3 plots the \( R \) value as a function of L/P for various lipids. DAC-Aβ exhibited strong binding only to GM1-containing membranes (open circles). The \( R \) value was almost saturated; i.e. the peptide was completely bound at an L/P of >200. Negatively charged PS (open diamonds), zwitterionic PC (open squares), or PC/cholesterol (2:1) (closed squares) bilayers did not bind the amyloid even at the highest L/P ratio examined (2550).

Cholesterol-induced Aβ Binding—The effects of lipid composition on peptide binding to GM1-containing DIG-mimicking membranes were examined in detail. Fig. 4A shows the \( R \) value as a function of GM1/DAC-Aβ ratio instead of L/P, because GM1 seems to constitute a “binding site” for the peptide. The binding was strongly dependent on GM1 as well as cholesterol contents. For the cholesterol-rich systems, a decrease in GM1 content from 40 to 20% significantly reduced DAC-Aβ binding (Fig. 4, open circles versus open triangles). Surprisingly, in the case of the GM1-poor systems, a decrease in the cholesterol/SM ratio...
b (2 ml) was titrated with aliquots of an unlabeled A mixed with a DAC-A in 10 mM Tris, 150 mM NaCl, 2 mM CaCl$_2$ buffer, pH 7.4. S.D. values for 2–4 preparations are shown by error bars at the peaks. B, LUVs (20 µM) composed of GM1/cholesterol/SM (40:30:30) were mixed with a DAC-A solution in the buffer (0.16 µM). Under this condition, the binding sites were almost saturated with DAC-A. The mixture (2 ml) was titrated with aliquots of an unlabeled A$_{-}$ (1–40) solution. Fluorescence intensity at 470 nm (excitation at 430 nm) was plotted as a function of DAC-A/unlabeled A$_{-}$ ratio (closed circles). The open circle shows the result of the converse experiment in which the vesicles were pretreated with excess unlabeled peptide and then DAC-A$_{-}$ was added. Error bars for duplicated measurements are within the symbols.

**Fig. 2. Detection of membrane binding of DAC-A$_{-}$** A, fluorescence emission spectra of 1 µM DAC-A$_{-}$ (excitation at 430 nm) were recorded in 10 mM Tris, 150 mM NaCl, 2 mM CaCl$_2$ buffer, pH 7.4 (trace 1) and in the presence of 80 µM GM1/cholesterol/SM (40:30:30) LUVs (trace 2) at 30 °C. S.D. values for 2–4 preparations are shown by error bars at the peaks. B, LUVs (20 µM) composed of GM1/cholesterol/SM (40:30:30) were mixed with a DAC-A$_{-}$ solution in the buffer (0.16 µM). Under this condition, the binding sites were almost saturated with DAC-A$_{-}$. The mixture (2 ml) was titrated with aliquots of an unlabeled A$_{-}$ (1–40) solution. Fluorescence intensity at 470 nm (excitation at 430 nm) was plotted as a function of DAC-A$_{-}$/unlabeled A$_{-}$ ratio (closed circles). The open circle shows the result of the converse experiment in which the vesicles were pretreated with excess unlabeled peptide and then DAC-A$_{-}$ was added. Error bars for duplicated measurements are within the symbols.

**Fig. 3. Lipid specificity of DAC-A$_{-}$ binding.** DAC-A$_{-}$ (1 µM) buffer solutions were titrated with LUVs of various lipid compositions at 30 °C, and fluorescence spectra were measured as in Fig. 2. Relative fluorescence enhancement, $r = (I - F) / F$, is plotted as a function of L/P. Fluorescence intensity at 466 nm in the presence and absence of LUVs are denoted by $F$ and $F$, respectively. Lipid composition was as follows: GM1/cholesterol/SM (40:30:30) (C), PC (C), PC/cholesterol (2:1) (■), PS (○). For PS preparations, CaCl$_2$ in the buffer was replaced by 1 mM EDTA. S.D. values for 2–4 preparations are shown by error bars.

**Fig. 4. Binding of DAC-A$_{-}$ to GM1-containing membranes.** A, R versus GM1/DAC-A$_{-}$ ratio plots are shown for GM1/cholesterol/SM (40:30:30) (○), GM1/cholesterol/SM (40:12:48) (■), GM1/cholesterol/SM (20:16:64) (▲), and GM1/cholesterol/SM (20:40:40) (△). S.D. values for 2–4 preparations are shown by error bars. B, binding isotherms were estimated from the data in A. The bound peptide per exofacial GM1, $x$, is plotted as a function of free peptide concentration, $c_f$. The traces are the best fit binding isotherms using Equation 4 and the parameters shown in Table I.

ratio from 1 to 0.25 markedly reduced DAC-A$_{-}$ binding (Fig. 4, open versus closed triangles).

Binding isotherms were obtained from Fig. 4A as follows. $R_{\text{max}}$ values were estimated by linear extrapolation of $R$ versus DAC-A$_{-}$/GM1 plots (DAC-A$_{-}$/GM1 → 0) and are summarized in Table I. In the case of the GM1-poor cholesterol-poor bilayers, the $R_{\text{max}}$ value was assumed to be the same as that of the GM1-poor cholesterol-rich system because data close to saturation could not be obtained (Fig. 4A). This assumption was reasonable because the $R_{\text{max}}$ values for the other systems were similar. The $R/R_{\text{max}}$ ratio gives the bound fraction of the peptide at each data point. Fig. 4B shows binding isotherms, i.e., the bound DAC-A$_{-}$ per exofacial GM1 (x) versus free DAC-A$_{-}$ concentration ($c_f$) plots. GM1 molecules on the outer leaflets (50% of total GM1) were assumed to be available for DAC-A$_{-}$ binding. The two isotherms of the GM1-rich systems were sigmoidal, implying cooperative binding. Therefore, the curves were analyzed by Equation 4 (Fowler’s equation) instead of the conventional Langmuir equation (26).

$$\theta = x / x_{\text{max}} = \left( \frac{1}{K} \exp(a \theta c_f) \right) / \left( 1 + K \exp(a \theta c_f) \right)$$

(Eq. 4)

The maximal $x$, $x_{\text{max}}$, were estimated by linear extrapolation of $x$ versus $1/c_f$ plots ($1/c_f \rightarrow 0$). The binding constant and lateral interaction parameter are denoted by $K$ (m$^{-1}$) and $a$, respectively. The binding isotherms at the lower GM1 content could be well explained by the Langmuir equation (i.e., $a = 0$ in Equation 4). The obtained binding parameters are summarized in Table I. Interestingly, the binding affinities ($K$) were very similar (2–3 × 10$^{-3}$ m$^{-1}$), whereas the binding capacities ($x_{\text{max}}$) were highly dependent on GM1 as well as cholesterol contents.

**Detection of GM1 Cluster—Excimer formation of BODIPY-GM1** GM1 was utilized to detect the GM1 cluster. The fluorophore BODIPY is known to form an excimer that emits red-shifted fluorescence (~630 nm) compared with monomer (~520 nm) (27). The excimer formation, which occurs upon collision of two dye molecules (one is in the excited state), is facilitated by higher local concentration of the dye as well as lower membrane rigidity. Fig. 5 shows the fluorescence emission spectra of BODIPY-GM1-labeled liposomes. The spectra are normalized to the monomer peaks because the excimer/monomer fluorescence ratio is directly proportional to local dye concentration (28). BODIPY-GM1/GM1/cholesterol/SM (10:30:30:30) liposomes corresponding to the GM1-rich cholesterol-rich system exhibited a large excimer fluorescence (trace 1). In contrast, BODIPY-GM1/PC (10:90) liposomes with the identical dye concentration showed much weaker excimer fluorescence (trace 2). The anisotropy value of monomer fluorescence ($r$) as a measure of membrane rigidity of the latter membrane (0.046) was significantly smaller than that of the former (0.104). Therefore, BODIPY-GM1 forms an excimer much more easily in the former DIG-like environment despite its higher rigidity (higher anisotropy), strongly indicating that GM1 is present in a locally concentrated state, i.e., in a cluster.

A reduction in total GM1 concentration from 40 to 20% markedly decreased excimer fluorescence (BODIPY-GM1/ GM1/cholesterol/SM = 5:15:40:40, corresponding to the GM1-
poor cholesterol-rich system, trace 3), although the intensity was much greater than that of the control BODIPY-GM1/PC (5:95) system (trace 5). The \(\tau\) value of the former (0.119) was again greater than that of the latter (0.041). It should be noted that the BODIPY-GM1/GM1 ratio remained constant at 1:3 in both GM1 40 and 20% systems. Therefore, if all GM1 molecules had been involved in the cluster formation, the same eximer fluorescence would have been observed. The weaker eximer fluorescence in the GM1-poor system indicated that the extent of clustering was smaller. BODIPY-GM1/GM1/cholesterol/SM (5:15:40:40) (trace 4), BODIPY-GM1/GM1/cholesterol/SM (5:15:16:64) (trace 3), BODIPY-GM1/GM1/cholesterol/SM (5:15:16:64) (trace 4), BODIPY-GM1/GM1/cholesterol/SM (5:15:16:64) (trace 5). The spectra are the averages for two preparations, and the S.E. values were within 700 degrees cm² dmol⁻¹ at 220 nm.

**DISCUSSION**

**Dye Labeling—**\(\alpha\)-peptides with slight chemical modifications, such as \(^{125}\text{I}\) (30–32) and Trp labeling (14), have been widely used in many studies and provided valuable information. The DAC moiety employed in this study was as small as a single aromatic amino acid and was attached to the N terminus of \(\alpha\)-peptide. First, DAC-\(\alpha\)A showed the common binding site with native \(\alpha\)A (Fig. 2B). Second, the interfacial location of the DAC moiety in membranes is fully compatible with the observation that native \(\alpha\)A lies on the surface of GM1-containing membranes (13). Third, DAC-\(\alpha\)A showed lipid specificity identical to that of the native peptide, as described below.

**Lipid Specificity—**DAC-\(\alpha\)-peptides showed no affinity for phospholipids PC or PS but high affinities for GM1-containing membranes at physiological ionic strength (Fig. 3), consistent with previous studies using native (11, 13) and Trp-labeled \(\alpha\)A (14). Our study clearly indicated that \(\alpha\)B does not bind cholesterol, because (i) DAC-\(\alpha\)B showed no binding activity (Fig. 2B) and (ii) The cholesterol content in DIG-mimicking membranes did not correlate with \(\alpha\)B-binding activity (Fig. 4). Wood's group (33) reported that aggregated but not freshly dissolved \(\alpha\)A binds cholesterol, in accordance with our results.

**Binding Isotherms—**Binding of peptides to lipid bilayers has often been analyzed by a simple partition model that does not include the concept of binding sites, because in most cases the driving force of peptide binding is not specific molecular recognition but simple electrostatic and hydrophobic interactions (34–36). However, \(\alpha\)A obviously recognizes GM1 or more accurately gangliosides (10–14). Therefore, the binding isotherms were analyzed by the cooperative binding model (Equation 4). The binding affinities, \(K\), were practically the same (2–3 \(\times 10^6\) M⁻¹) for the four DIG-mimicking systems investigated (Table 1). The competitive binding experiments (Fig. 2B) suggested that the \(K\) value of native \(\alpha\)A is 40-fold smaller (\(\approx 6 \times 10^4\) M⁻¹), which corresponds to a difference in a Gibbs free energy of 2 kcal/mol. This value is a reasonable one for transfer of the DAC moiety from water to membrane interface (25). A binding af-

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**TABLE I**

| System                                             | Lipid composition          | \(R_{\text{max}}\) \(\times 10^5\) | \(K\) \(\times 10^6\) | \(\sigma\) | \(\sigma_{\text{max}}\) |
|----------------------------------------------------|----------------------------|------------------------------------|------------------------|---------|------------------------|
| GM1-rich cholesterol-rich GM1/cholesterol/SM (40:30:30) | GM1/cholesterol/SM (40:30:30) | 15.8 ± 0.2                         | 2.4 ± 0.1              | 1.9 ± 0.1 | 0.057 ± 0.001           |
| GM1-rich cholesterol-poor GM1/cholesterol/SM (40:12:48) | GM1/cholesterol/SM (40:12:48) | 16.2 ± 0.3                         | 1.7 ± 0.3              | 1.9 ± 0.2 | 0.060 ± 0.001           |
| GM1-poor cholesterol-rich GM1/cholesterol/SM (20:40:40) | GM1/cholesterol/SM (20:40:40) | 18.5 ± 0.5                         | 2.9 ± 0.4              | 0°       | 0.019 ± 0.001           |
| GM1-poor cholesterol-poor GM1/cholesterol/SM (20:16:64) | GM1/cholesterol/SM (20:16:64) | 18.5°                              | 3.0 ± 2.9°             | 0°       | 0.001 ± 0.000           |

* Binding parameters from Equation 4 are the means ± S.D. obtained on 2–4 independent measurements.
* Langmuir type (noncooperative binding) analysis was performed.
* Assumed to be the same as that of the GM1-poor cholesterol-rich system.
* The large error is due to the absence of data points close to the origin (Fig. 4B).
finity of $7 \times 10^5$ m$^{-1}$ was reported for the Y10W-Aβ(1–40)-GM1 system (14). The Tyr-to-Trp substitution enhances membrane binding by $\sim 1$ kcal/mol (25), which corresponds to a 5-fold increase in affinity. Therefore, taken together with the present estimation, the affinity of native Aβ for GM1 is estimated to be $\sim 10^5$ m$^{-1}$. Even in the absence of specific molecular recognition, binding isotherms are fitted by the Langmuir equation. The $K$ values for magainin 1-PS (37) and calcitomin/dimyristoylphosphatidylglycerol (38) systems were reported to be $3.8 \times 10^8$ and $1 \times 10^8$ m$^{-1}$, respectively, which were comparable with the estimated affinity of native Aβ.

If individual GM1 molecules constitute binding sites for Aβ, the binding isotherms (Fig. 4B) would be independent of lipid composition. However, the binding capacity increased quadratically with intramembrane GM1 content in the cholesterol-rich matrix (Fig. 4B and Table I), suggesting that some cooperative interactions between GM1 molecules generate the binding site. In accordance with this view, the membrane binding of native Aβ(1–40) also requires a threshold GM1 content dependent on the lipid composition of the host matrix (12, 13). The most straightforward interpretation is that Aβ recognizes not monomeric but clustered GM1 or that in a GM1-enriched microdomain (39), the formation of which is regulated by cholesterol content. Indeed, a correlation was observed between binding capacity ($x_{\text{max}}$ in Table I) and excimer formation of BODIPY-GM1 as a measure of clustering (Fig. 5). However, the relationship between $x_{\text{max}}$ and excimer fluorescence was semi-quantitative. The $x_{\text{max}}$ value of the GM1-poor cholesterol-poor system was very small, whereas significant excimer fluorescence was observed (Fig. 5, trace 4), probably because the introduction of relatively high amounts of BODIPY-GM1 (25% of total GM1) slightly affected domain formation. The excimer experiments also suggested that DIG-like environments play a crucial role in GM1 clustering. Ferraretto et al. reported that GM1 as well as cholesterol form GM1- and cholesterol-enriched domains in SM bilayers (39). It is therefore plausible that at lower GM1 contents (e.g., 20%) an increase in cholesterol content, through segregation of cholesterol molecules by the cholesterol-rich domain formation, enhances local GM1 concentration, which leads to GM1 clustering (Fig. 4). In contrast, a GM1 content of 40% appears to be sufficient for effective formation of GM1-rich domains, and thus cholesterol content would have no further effect on GM1 clustering. In the PC matrix, a high level of excimer fluorescence was never observed despite its lower rigidity (Fig. 5, traces 2 and 5). An electron microscopic study also indicated that GM1 is molecularly dispersed in fluid phosphatidylcholine bilayers at lower GM1 contents (40).

Cooperativity was observed for peptide binding to the GM1-rich membranes with larger capacities. This may be related to conformational transition from $\alpha$-helix-rich conformations at lower $x$ values to $\beta$-sheet-rich conformations at higher $x$ values (Fig. 6). The formation of the latter structures can involve interpeptide interactions. Terzi et al. (41) reported similar structural transition in phosphatidylglycerol bilayers at low ionic strength. The presence of $\alpha / \beta$ structures was also found in ganglioside-containing membranes (10). The conformational transition of the N-terminal region (residues 10–24) from $\alpha$-helix to $\beta$-strand was reported to facilitate amyloid formation (42).

**Pathological Implications**—Recently, a great deal of attention has been focused on the pathological implications of altered cholesterol metabolism, which is likely to occur with aging or the expression of apolipoprotein E, in the development of AD. There is accumulating evidence that the metabolism of amyloid precursor protein, including Aβ generation, is significantly modulated by the content of cellular cholesterol (43–45). It is particularly interesting that a recent study indicated a unique Aβ species with seeding ability was generated by cultured cells in a cholesterol-dependent manner (46). Even with this information we are still far from understanding how cholesterol is involved in the development of AD, especially in the initiation of amyloid fibril formation.

The results of the present study indicated, for the first time, that increases in the content of cholesterol in the membrane induce the formation of GM1/Aβ, one of the candidates as an endogenous seed for Alzheimer amyloid. With regard to the alteration of cholesterol in neuronal membranes, recent studies by Wood and co-workers (47, 48) are very informative. They reported that the content of cholesterol in the exofacial leaflets of synaptic plasma membrane is increased during aging (47) and by apolipoprotein E deficiency (48). Taken together with the results of present study, these observations strongly suggest that alterations in the content of cholesterol in neuronal membranes underlie abnormal aggregation of Aβ in the AD brain.

Finally, if Aβ forms amyloid fibrils via seeded polymerization in the brain with AD, then the seed could be a target for therapeutic and preventive treatment regimens for AD. Indeed, we have recently found that GM1/Aβ formed in DIG-like membranes works as a seed for fibril formation. To generate a compound that specifically recognizes GM1/Aβ and inhibits its seeding ability, it will be necessary to clarify the molecular processes underlying alterations of the secondary structures of Aβ via binding to and accumulation in GM1 “clusters.”

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