The interaction between Alzheimer amyloid β(1–40) peptide and ganglioside G_{M1}-containing membranes

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Received 25 November 1996; revised version received 12 December 1996

Abstract The interaction between Alzheimer amyloid peptide Aβ(1–40) and membrane lipids was studied by circular dichroism spectroscopy under the conditions of physiologically relevant ionic strength and neutral pH. The peptide binds to the membranes containing ganglioside G_{M1} and upon binding undergoes a conformational transition from random coil to an ordered structure rich in β-sheet. This interaction appears to be ganglioside-specific as no changes in Aβ(1–40) conformation were found in the presence of various phospholipids or sphingomyelin. The isolated oligosaccharide moiety of the ganglioside was ineffective in inducing alterations in the secondary structure of Aβ(1–40). No interaction was observed between ganglioside G_{M1} and the N-terminal peptide fragment Aβ(1–28). Binding to the ganglioside is likely to modulate the neurotoxic and/or amyloidogenic properties of Aβ(1–40).

Key words: β-Amyloid peptide; Alzheimer's disease; Ganglioside G_{M1}; Peptide conformation

1. Introduction

A key pathological feature of Alzheimer's disease (AD) is the formation and progressive deposition of insoluble amyloid fibrils in brain parenchyma and in the walls of cerebral and meningeal blood vessels [1]. The principal component of amyloid deposits is the ~4 kDa amyloid β-peptide (Aβ) [2,3], a product of proteolytic processing of a much larger amyloid precursor protein (APP) encoded by a gene on chromosome 21 [4]. The role of Aβ in the pathogenesis of AD is suggested by the findings that specific mutations in APP (in close proximity to the amino- or carboxyl-terminus of Aβ or within the Aβ region) are linked to familial forms of AD [5–7]. A link between Aβ and AD neuropathological lesions is also indicated by the findings that fibrillar Aβ is toxic to neuronal cells in culture [8–10]. However, the molecular mechanisms of the neurotoxic action of the peptide remain largely unknown.

A growing number of observations indicate that Aβ may alter the physicochemical properties of neuronal membranes, including membrane fluidity [11] and the permeability to ions and nonelectrolytes [12–18]. These findings strongly suggest that at least some of the pathophysiological effects of Aβ may be mediated by peptide-membrane interactions. Interest in the interaction between Aβ and the constituents of brain membranes has been further stimulated by the recent identification of a ganglioside G_{M1}-bound form of Aβ as an important component of early diffuse plaques in AD brain [19]. In this communication, we explore the molecular mechanism of the interaction between Aβ and the lipid components of biological membranes. Using circular dichroism spectroscopy, our data demonstrate that Aβ(1–40) binds to model membranes containing ganglioside G_{M1} and upon binding undergoes a major conformational transition. This effect appears to be ganglioside-specific as under the conditions of physiologically relevant ionic strength, no changes in Aβ conformation could be detected in the presence of ganglioside-free vesicles composed of zwitterionic or acidic phospholipids.

2. Materials and methods

2.1. Materials

The peptides were obtained from Bachem California Inc. (Torrance, CA) [Aβ(1–40), lot 506063] or American Peptide Company (Sunnyvale, CA) [Aβ(1–40), lot K01047A1; Aβ(1–28), lot H02022T1]. The purity of the peptides was greater than 98%. 1-Palmitoyl-2-oleoyl phosphatidylcholine, 1-palmitoyl-2-oleoyl phosphatidylglycerol and 1-palmitoyl-2-oleoyl phosphatidylserine were purchased from Avanti Polar Lipids (Alabaster, AL). Ganglioside G_{M1} was obtained from Calbiochem (La Jolla, CA) and sphingomyelin from Sigma (St. Louis, MO). The pentasaccharide portion of ganglioside G_{M1} (II^3NeuAc-GgOse$_4$) was obtained from BioCarb Chemicals (Lund, Sweden). Prior to the experiments, the peptides were dissolved to 1 mg/ml in HFIP (Sigma) and stored at −20°C. As reported previously [20], the treatment with HFIP breaks up any aggregates of Aβ and thus allows for normalization of the properties of different preparations of the peptide.

2.2. Preparation of lipid vesicles

To obtain small unilamellar vesicles, lipid films of a desired composition were dispersed by vortexing in 10 mM phosphate buffer, pH 7.4. The resulting mixture of multilamellar liposomes was then sonicated in an ice bath for approximately 15 min using a probe-type sonifier. Metal debris from the titanium tip of the probe was removed by centrifugation for several minutes in a microfuge. Vesicles were kept at room temperature and used within a few hours after preparation.

2.3. Circular dichroism spectroscopy

Aqueous peptide solutions for circular dichroism measurements were prepared by evaporating the organic solvent from the stock peptide solution in HFIP and redissolving the resulting peptide film in buffer. The solutions were used within 3 h after preparation. The interaction of Aβ peptides with lipids was studied by following changes in circular dichroism spectra upon the addition of small aliquots of a suspension of lipid vesicles or micellar ganglioside to 25 μM peptide solution in buffer. Circular dichroism spectra were acquired on a JASCO J-600 spectropolarimeter at room temperature using a 1 mm quartz cell. Typically, four spectra were averaged and smoothed to improve signal to noise ratio. To correct for the contribution of lipid to circular dichroism spectra, the signal of pure lipid at appropriate concentration was subtracted from the signal of peptide-lipid samples. This method of subtracting the lipid contribution was valid since for those compounds that possess strong CD signal but failed to interact with the peptide (sphingomyelin, G_{M1}-oligosacchar-

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Abbreviations: AD, Alzheimer's disease; POPC, 1-palmitoyl-2-oleoyl phosphatidylcholine; POPG, 1-palmitoyl-2-oleoyl phosphatidylglycerol; POPS, 1-palmitoyl-2-oleoyl phosphatidylserine; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; CD, circular dichroism

PII S0014-5793(96)01504-9

ide), subtraction yielded essentially an identical spectrum to that of the peptide alone (see Section 3).

3. Results

The effect of various lipids on the backbone conformation of Aβ peptides was studied by far-UV circular dichroism spectroscopy. The spectrum of a freshly prepared solution of Aβ(1–40) in 10 mM phosphate buffer has a minimum at approximately 197 nm and is characteristic of an essentially random conformation [21]. Under the conditions of our experiments, the above spectrum remained unchanged for at least 8 h, indicating the preservation of an unordered (monomeric) structure of the peptide. Upon solubilization in water, ganglioside G_{M1} is known to form micelles [22]. Addition of micellar ganglioside to the solution of Aβ(1–40) resulted in a rapid change in the peptide CD spectrum (Fig. 1A). The effect was concentration dependent and appeared to saturate at a ganglioside to peptide molar ratio of approximately 20:1 (accurate determination of the saturation point was hampered by a rapid deterioration of the signal to noise ratio in CD spectra at high lipid concentration). The spectrum of Aβ(1–40) in the presence of a 18-fold molar excess of ganglioside G_{M1} has a minimum at approximately 217 nm and a maximum at 197 nm, the features characteristic of an ordered, predominantly β-sheet conformation [21]. The above spectrum remained unchanged for many hours after formation of the peptide-lipid complex. In order to assess the role of electrostatic effects in the interaction between Aβ(1–40) and ganglioside G_{M1}, the circular dichroism measurements described above were repeated in the presence of 100 mM NaF (sodium fluoride was used instead of NaCl to avoid problems associated with high far-UV absorption of the latter salt). As shown in Fig. 1, very similar ganglioside-induced transitions in Aβ conformation were observed in the presence and absence of NaF. Similar results were also obtained in a buffer containing 100 mM sodium phosphate (data not shown for brevity). Furthermore, the spectrum characteristic of ganglioside G_{M1}-bound peptide was preserved upon increasing the salt concentration to 200 mM.

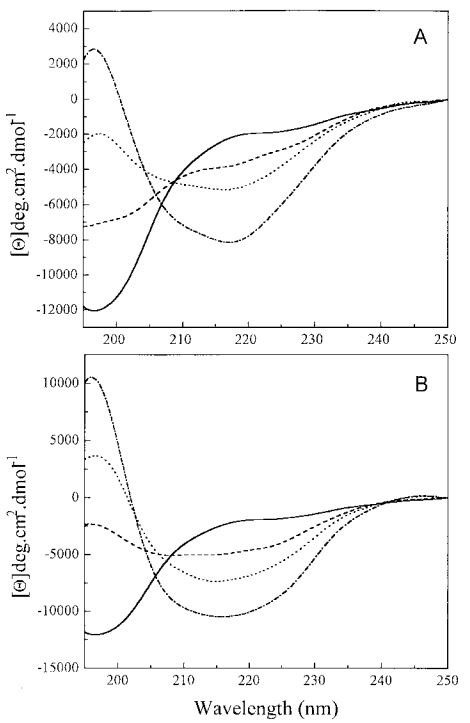

Fig. 1. Circular dichroism spectra of 25 μM Aβ(1–40) alone (solid line) and in the presence of various concentrations of ganglioside G_{M1}. The ganglioside:peptide molar ratios are: 5:1 (dashed line), 10:1 (dotted line) and 18:1 (dash-dotted line). A: 10 mM phosphate buffer, pH 7.4. B: 10 mM phosphate buffer, 100 mM NaF, pH 7.4. Spectra were corrected by subtracting the contribution of lipid alone.

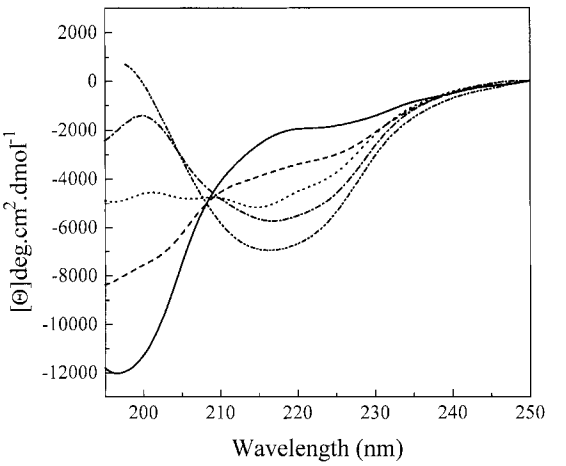

Fig. 2. Circular dichroism spectra of 25 μM Aβ(1–40) alone (solid line) and in the presence of POPC:ganglioside G_{M1} (3:1) vesicles. The ganglioside:peptide molar ratios are: 2.5:1 (dashed line), 5:1 (dotted line), 7:1 (dash-dotted line) and 9:1 (dash-double dotted line). The measurements were performed in 10 mM phosphate buffer, 100 mM NaF, pH 7.4. Spectra were corrected by subtracting the contribution of lipid alone.

Changes in CD spectra indicative of the unordered structure → β-sheet transition were also observed when Aβ(1–40), in high ionic strength buffer (10 mM phosphate, 100 mM NaF), was titrated with unilamellar vesicles composed of POPC and 25 mol% of ganglioside G_{M1} (Fig. 2). The above effect is apparently ganglioside-specific as no changes in the peptide CD spectrum (in the same buffer) were detected in the presence of ganglioside-free POPC membranes, vesicles composed of acidic phospholipids such as POPG and POPS, or those containing sphingomyelin (spectra not shown for brevity). An alternative procedure was also used to increase the ganglioside to peptide molar ratio. In these experiments, different aliquots of Aβ(1–40) solution were incubated with vesicles containing a constant amount of POPC and increasing proportions of ganglioside G_{M1}. At corresponding ganglioside to peptide ratios, results essentially identical to those shown in Fig. 2 were obtained (data not shown). Furthermore, no unordered → β-sheet transition occurred in the presence of even a large molar excess of the isolated oligosaccharide moiety of ganglioside G_{M1} (Fig. 3). This clearly indicates that both the sugar head group and the hydrocarbon portion of the ganglioside are required to bring about a conformational transition in Aβ(1–40).

Aβ(1–40) has an amphiphilic character. The N-terminal portion of the peptide is relatively polar and contains a number of charged residues, while the C-terminal region is highly hydrophobic. In order to determine which part of Aβ(1–40) is involved in ganglioside G_{M1} binding, the experiments described above were repeated with the peptide fragment

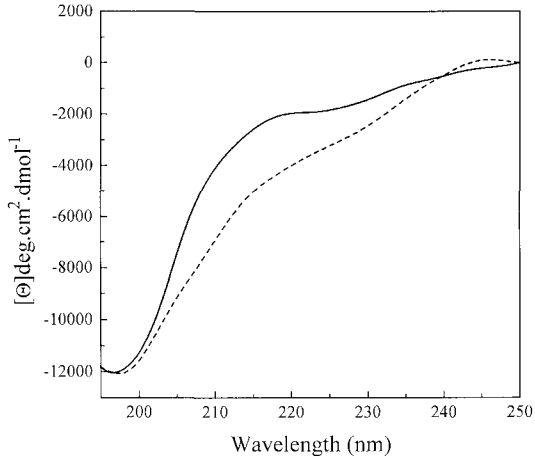

Fig. 3. Circular dichroism spectra of 25 μM Aβ(1–40) alone (solid line) and in the presence of G_{M1}-oligosaccharide at an oligosaccharide:peptide molar ratio of 19:1 (dashed line). The measurements were performed in 10 mM phosphate buffer, 100 mM NaF, pH 7.4. Spectra were corrected by subtracting the contribution of the oligosaccharide alone.

Aβ(1–28). Previous studies have demonstrated that the fragment Aβ(1–28) forms amyloid-like fibrils of a morphology similar to those found in AD brain [23,24]. As shown in Fig. 4, the addition of ganglioside G_{M1} to Aβ(1–28) solution in buffer (10 mM phosphate, 100 mM NaF) did not result in any changes in the CD spectrum of the peptide.

4. Discussion

Apparently conflicting reports have been published regarding the CD spectra of soluble Aβ(1–40) in aqueous buffer. While some authors have reported spectra indicative of a mixture of random coil and β-sheet conformations [25,26], the spectra obtained by others are characteristic of an essentially pure random coil structure [27,28]. These discrepancies likely reflect different propensities of various peptide preparations to self-associate (e.g. as a result of the presence of different amounts of aggregated 'seeds' which promote the self-association process [29,30]). Indeed, our present results show that upon complete disaggregation of the peptide by HFIP treatment [20], Aβ(1–40) at low concentration in aqueous solution adopts a random conformation with no indication of β-sheet structure. The above conformation (and thus the monomeric state of the peptide) remains stable for many hours.

The key observation of the present study is the finding that Aβ(1–40) binds to micellar ganglioside G_{M1} or ganglioside G_{M1}-containing membrane vesicles, and as a result of this binding, undergoes a rapid transition to an ordered conformation characterized by a high content of β-sheet structure. A similar conformational transition of Aβ(1–40) was recently observed in the presence of the model acidic phospholipid phosphatidylglycerol [28]. However, peptide binding to the latter lipid appears to be purely electrostatic; it is completely inhibited in the presence of 100 mM NaCl [28] or 100 mM NaF. This may be contrasted with the interaction between Aβ(1–40) and ganglioside G_{M1} which shows very little sensitivity to the ionic strength of the buffer. The notion that electrostatic effects do not play a dominant role in Aβ(1–40)-ganglioside binding is further indicated by the experiments with a highly charged peptide fragment Aβ(1–28). The apparent lack of interaction between the latter peptide and ganglioside G_{M1} suggests that the apolar C-terminal region of Aβ(1–40) is crucial for ganglioside binding.

We have also tested a number of other lipids, including acidic phosphatidylserine as well as zwitterionic phosphatidylcholine and sphingomyelin. Under the conditions of physiologically relevant ionic strength (100 mM NaF or 100 mM sodium phosphate), none of the above lipids was able to induce any conformational transition in Aβ(1–40). Therefore, the effect described in this study appears to be ganglioside-specific. The roles of individual chemical groups of ganglioside molecule in the interaction with Aβ remain to be elucidated; some light in this respect should be shed by the ongoing experiments with ganglioside variants of different head group structure. Nevertheless, it is striking that no changes in Aβ(1–40) conformation could be detected in the presence of the isolated oligosaccharide moiety of ganglioside G_{M1}. This strongly suggests that the membrane (or micellar) surface is required to bring about a conformational transition in the peptide. The transition to an ordered (largely β-sheet) structure may be facilitated by the interfacial environment of the lipid head group region, surface-induced aggregation of the peptide, partial penetration into the hydrocarbon region, or the combination of these factors.

The present findings are likely to be of direct relevance to the neurotoxic action of Aβ. While present only in relatively small quantities in most tissues, gangliosides are abundant components of nervous tissue [31,32]. Ganglioside G_{M1} tends to accumulate in certain regions of neurons, especially in synaptic membranes [32,33]. We propose that the specific interaction of Aβ with ganglioside-rich domains of neuronal membranes may result in a local membrane perturbation. This, in turn, could trigger a cascade of events that eventually lead to neuronal cell death. Another potential role of gangliosides in the pathology of AD has been recently suggested by Yanigasawa et al. [19] and Selkoe [34]. Based on the observation that ganglioside G_{M1}-bound Aβ is associated with early diffuse plaques [19], these authors have hypothesized that ganglioside-bound peptide may act as a 'seed' that facilitates forma-

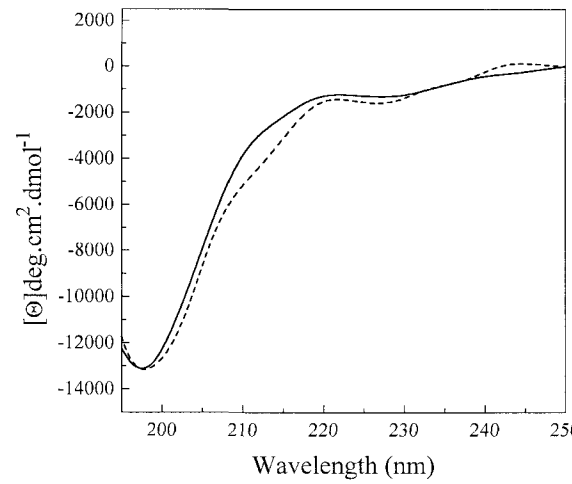

Fig. 4. Circular dichroism spectra of 25 μM Aβ(1–28) (solid line) alone and in the presence of ganglioside GM_1 at a ganglioside:peptide molar ratio of 18:1 (dashed line). The measurements were performed in 10 mM phosphate buffer, 100 mM NaF, pH 7.4. The spectrum in the presence of ganglioside G_{M1} was corrected by subtracting the contribution of lipid alone.

tion of a mature amyloid plaque. The above hypothesis remains to be tested. Nevertheless, the finding that ganglioside G_{M1} binding to Aβ results in peptide conformational transition to a (presumably intermolecular) β-sheet structure is strongly supportive of the general idea that gangliosides may play an important role by modulating the amyloidogenic properties of Aβ.

Acknowledgements: This work was supported in part by the Britton's Fund.

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