Study of the Correlation of Secondary Structure of Beta-Amyloid Peptide (Aβ40) with the Hydrophobic Exposure under Different Conditions

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Abstract. Aβ is the core protein of extracellular plaque of Alzheimer's disease, and its neurotoxicity is relative to its conformation. In the current work, the effects of various factors, such as pH, ionic strength and lipid membranes, on the secondary structure of Aβ were studied by circular dichroism. In addition, we detected the exposure of hydrophobic sites of Aβ under different conditions using ANS fluorescence. The results showed that the hydrophobic exposure of the protein was correlated with the content of β-sheet conformation in the phospholipid-containing environment. The β-sheet content and hydrophobic exposure of Aβ both increased when reacted with pure PC vesicles, while no β-sheet content and very low hydrophobic exposure were detected after reaction with 30 % cholesterol containing PC vesicles. Since β-sheet conformation is considered as the toxic conformation of Aβ, such correlation may be important for the pathology of AD.

Key words: β-amyloid peptide — Vesicle — Circular dichroism — ANS fluorescence

Introduction

Alzheimer's disease (AD) is a slowly developed, neurodegenerative disease, which would damage memory and cognitive ability and is characterized as the existence of extracellular amyloid plaques and cerebrovascular deposits. The core protein of the amyloid plaques is β-amyloid peptide (Aβ) which adopts anti-parallel β-sheet conformation (Inouye et al. 1993). Aβ is considered as the normal product of neuron and non-neuronal cell, since it can be detected in cerebrospinal fluid (CSF) and amyloid plaques can be found in both normal aged and AD brains (Esch et al. 1990; Haass et al. 1992).

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Aβ is the mixture of multifold length peptides (39 to 43 amino acid residues), in which Aβ40 is the major component (about 50–70%) (Mori et al. 1992; Roher et al. 1993; Murphy et al. 1999). Some reports consider that Aβ42 is important to AD due to its relation with diffused, incompact plaques (Hardy 1997; Selkoe 1997). But as the major protein in senile plaques (Suzuki et al. 1994) and CSF, Aβ40 is also very important. For example, although Aβ42 is important to the early stage of AD, Aβ42 itself could not form the mature, toxic plaques if there was the continua aggradations of Aβ40 (Shin et al. 1997). In addition, the interaction between Aβ and neuronal membrane may influence the impermeability and fluidity of membrane, which may be important to the pathogenesis of AD (Racchi et al. 1997; Waiter et al. 1997; Mason et al. 1998; Martinez-Senac et al. 1999; Matsuzaki and Horikiri 1999; Eckert et al. 2000). Our previous work revealed that the membrane insertion of Aβ might provide a possible pathway that Aβ prevented itself from aggregation and fibril formation. In the current work the conformation and the exposure of hydrophobic sites of Aβ40 at different conditions, especially after reacted with vesicles, were studied to give some hints to the pathology of AD.

Materials and Methods

Materials

1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), cholesterol and anilino naftalen sulfonic acid (ANS) fluorescence probe were all purchased from Sigma Chemical Co. (St. Louis, MO, USA); β-amyloid peptide, Aβ40, was purchased from AnaSpec Co. (USA), whose purity (>95%) was analyzed by HPLC and checked by MALDI-TOF MS. And unless stated, Aβ refers to Aβ40 in the following text. All the other chemicals used were of analytical grade and manufactured in China. Usually the subphase buffer was 50 mmol/l Tris-HCl containing 25 mmol/l NaCl with pH 7.4.

Preparation of phospholipid vesicles

Small unilamellar vesicles (SUVs), giving the smallest scatter in circular dichroism (CD) measurement, were prepared as follows: lipids of the desired composition were mixed in chloroform/methanol (3 : 1, v/v) and dried under a stream of nitrogen. Residual solvents were removed under high vacuum for 5–7 hours. The lipid films were then resuspended and sonicated in the desired buffer to near optical clarity by using a probe sonicator. Suspension was centrifuged to get rid of the metal debris from the titanium tip. The concentration of phospholipid was determined by phosphate analysis (Ames 1966).

Circular dichroism spectroscopy

Circular dichroism (CD) measurements were carried out on a Jasco J-715 spectropolarimeter. Samples were scanned at least ten times at the rate of 200 nm/min with a 0.5 nm step, 1 nm bandwidth and then averaged. The path length of the

quartz cell was 2 mm. In the experiments, a blank run made with the vesicles or buffer alone was carefully subtracted from the experimental spectra for correction. The 200–250 nm spectra were used for analysis and calculation because in this wavelength range, the vesicles scattering had little effect on the CD spectra (Xia and Sui 2000). All spectra were smoothed and converted to the mean residue ellipticity, $[\theta]$ in deg·cm^2/dmol, by using mean residue molecular weight of 110. And the secondary structure of the peptide was estimated from spectral simulations based on reference CD spectra of Yang et al. (1986). Unless stated, the final concentration of Aβ was 23 μmol/l.

Hydrophobic fluorescence detection

ANS binding assays were used to detect the nonpolar residues exposed to solvent, so ANS, as a hydrophobic fluorescent probe, is used in studies of protein structure. ANS has very low fluorescence intensity in aqueous solution; however, in a nonpolar environment, it fluoresces strongly (Stryer 1965). This increase in the fluorescence intensity in the emission maximum is due to the transfer of the ANS molecule from a polar setting to a nonpolar region of the protein molecule. Fluorescence emission spectra were recorded for solutions held in quartz cuvettes (1 cm path length) with an Instruments of Hitachi F-2500 Fluorescence Spectrophotometer. For each spectrum, the excitation wavelength was set at 360 nm, and the emission spectrum was recorded every 0.5 nm from 400 to 550 nm with an integration time of 0.08 s at room temperature. The ANS detection was performed immediately after CD measurement and the samples were diluted to the final Aβ concentration of 2 μmol/l. Then ANS was added into solutions to obtain concentration of 20 μmol/l. In the experiments, a blank run made with buffer or vesicle solution alone with 20 μmol/l ANS was carefully subtracted from the experimental spectra for correction.

Results

The structure changes of Aβ in aqueous solution

CD spectra of Aβ with different protein concentration

The secondary conformation of Aβ with different protein concentration were measured and shown in Figure 1. The figure indicates that the protein concentration could effect the conformation of Aβ to some extent: Aβ mainly existed in random-coil at low concentration, while α-helix conformation increases along with concentration. In detail, the conformation of Aβ was 27.1% β-sheet without α-helix in 23 μmol/l Aβ buffer, and 7.4% α-helix and 34.8% β-sheet in 46 μmol/l Aβ buffer, and 20.1% α-helix and 25.9% β-sheet in 69 μmol/l Aβ buffer.

The effect of ion strength on the conformation of Aβ

Figure 2 showed the CD spectra of Aβ in 25 mmol/l, 50 mmol/l, 100 mmol/l and 200 mmol/l NaCl buffer. From the figure we can know that ion strength almost has

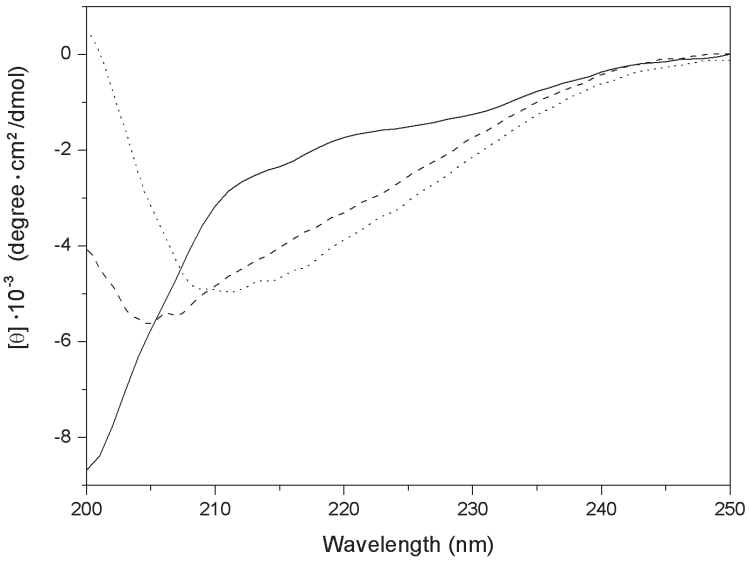

Figure 1. The conformation of Aβ in solution with the protein concentration of 23 μmol/l (——), 46 μmol/l (– – –) and 69 μmol/l (· · · · · ·). Buffer used in experiments was 50 mmol/l Tris-HCl containing 25 mmol/l NaCl with pH 7.4.

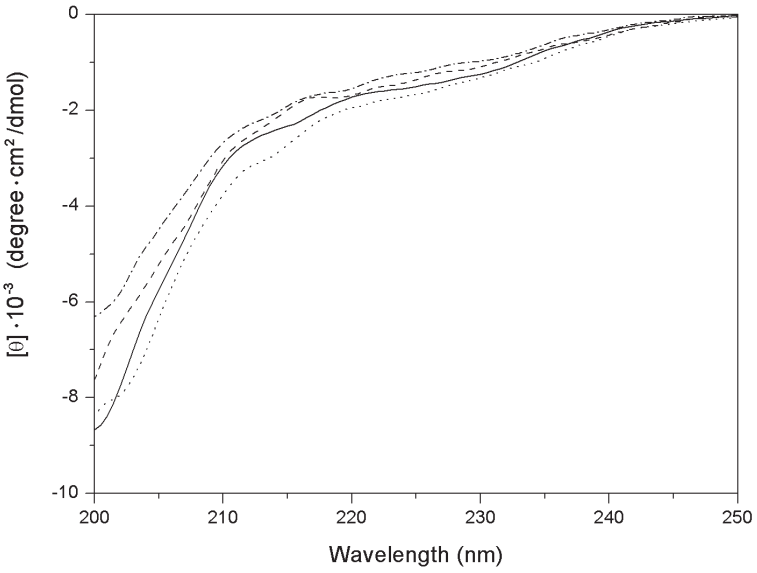

Figure 2. The effect of ion strength on the conformation of Aβ, and the Na^+ concentration used is 25 mmol/l (——), 50 mmol/l (– – –), 100 mmol/l (· · · · · ·) and 200 mmol/l (– · – ·). Buffer was similar to Figure 1, only different in concentration of Na^+.

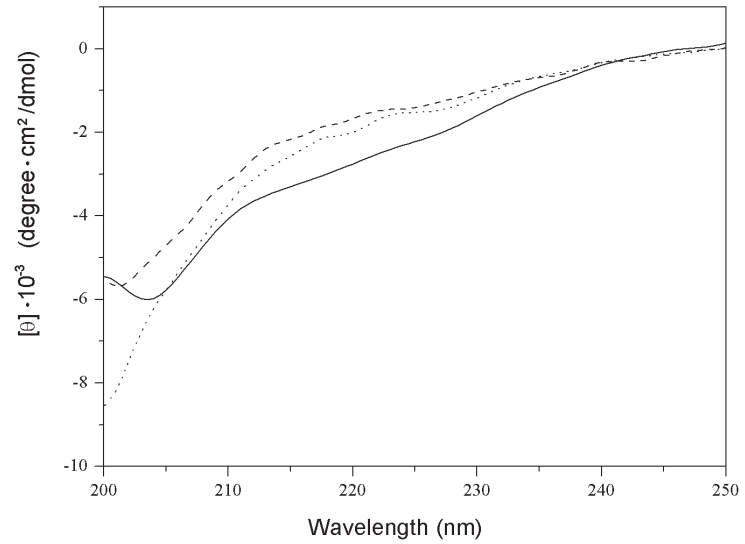

Figure 3. The effect of pH on the conformation of Aβ, showing the conformation of Aβ in pH 5.0 (——), pH 6.0 (– – –) and pH 7.0 (· · · · · ·). To supply enough range of pH, we used Na_2HPO_4-citric acid buffer.

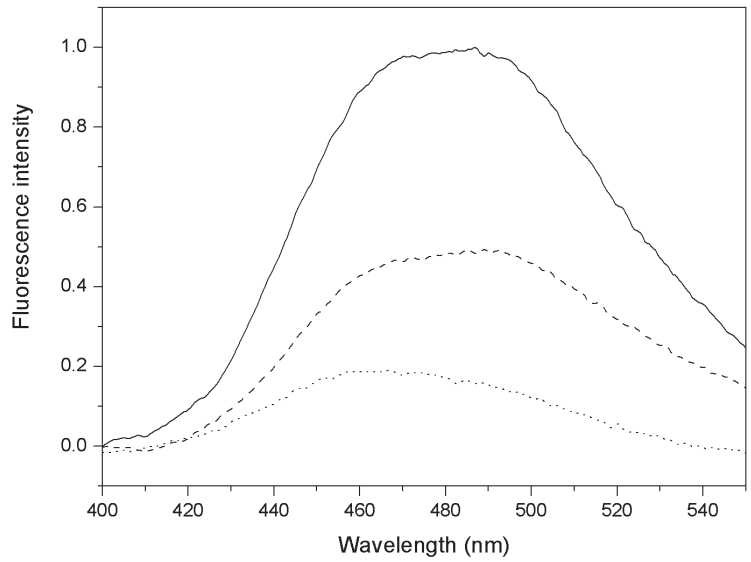

Figure 4. The exposure of hydrophobic sites of Aβ at different pH aqueous solution detected by ANS fluorescence with pH 5.0 (——), pH 6.0 (– – –) and pH 7.0 (· · · · · ·). The excitation wavelength for ANS was 360 nm and data of 400–550 nm emission wavelength were collected. The final concentration of Aβ was 2 μmol/l with 20 μmol/l ANS.

no influence on the conformation of Aβ: only a little more β-sheet content being observed in high ion strength buffer.

The effect of ion strength on the hydrophobic exposure of Aβ was also detected by ANS, a well known hydrophobic fluorescent probe. The results showed that the ANS hydrophobic fluorescence intensity had almost no change in different ion strength buffer (data not shown), indicating that ion strength also had little effect on the hydrophobic exposure of Aβ.

The effect of pH on the conformation of Aβ

The CD spectra in Figure 3 indicated that more β-sheet content of the conformation of Aβ was induced with the decrease of pH (in the range of 5.0–7.0) according to Yang's simulation. The results of ANS fluorescence detection were shown in Figure 4, which indicated that the fluorescence intensity increased greatly with the decrease of pH. That is, Aβ expose more hydrophobic sites in buffer with lower pH value.

The structure changes of Aβ in vesicle solutions

The effect of PC vesicles on the conformation of Aβ

Before CD measurement a pre-experiment was performed to determine the critical molar ratio of phospholipid for Aβ interaction, which is defined as the minimal ratio of lipid/Aβ for maximal interaction of Aβ with lipid vesicles. In the present case the critical molar ratio of lipid/Aβ of about 50 was obtained (data not shown). Thus in the following measurements we chose 100 as the actual lipid/Aβ ratio to assure enough of lipid.

Aβ incubated in the PC vesicle solution has been measured and showen in our previous paper (Ji et al. 2002a). The results showed that PC vesicle solution could induce a little more α-helix and β-sheet conformations (9.5% α-helix and 29% β-sheet) compared to that in aqueous solution. Since Aβ can not insert into pure PC bilayer (Ji et al. 2002a), the conformation change may be mainly induced by the different microenvironment of the membrane surface.

The effect of cholesterol containing vesicles on the conformation of Aβ

Cholesterol is an integral component of all eukaryotic cell membranes and is essential for normal cellular functions (Brown and Goldstein 1997; Howland et al. 1998). Within the cell cholesterol is not uniformly distributed. Plasma membrane contains the highest levels of cholesterol (nearly 90% of total cellular cholesterol) (Yeagle 1985). The location of Aβ in the sequence of amyloid precursor protein (APP) is in its trans-outer membrane part (Mills and Reiner 1999). Therefore the neutral cholesterol containing PC system could simulate the outer membranes of cell, which may be the membrane that Aβ can interact with.

The CD spectra of Aβ in different cholesterol containing PC vesicle solutions were measured and shown in Figure 5. The main phenomenon observed was that the content of β-sheet conformation was critically dependent on the cholesterol

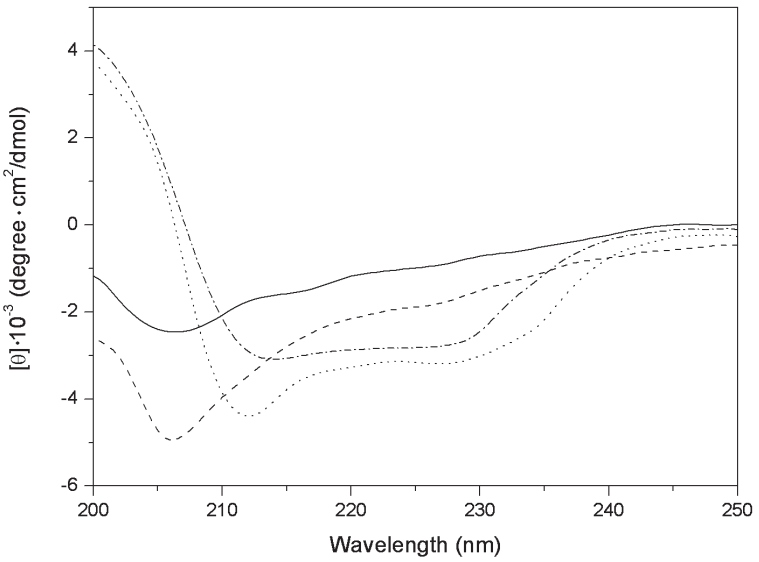

Figure 5. The effect of cholesterol containing PC vesicles on the conformation of Aβ with 20% cholesterol containing (– – –), 33% cholesterol containing (· · · · · ·) and 45% cholesterol containing PC vesicles (– · – ·) with a control of Aβ in pure PC vesicles (——). In a general experiment, a desired amount of vesicle solution, determined by the lipid/protein ratio of 100 was added to an Eppendorf tube, and then Aβ (1 mg/ml) was injected to acquire the final concentration of 23 μmol/l. After incubation for 90 min (providing enough time for the interaction between Aβ and vesicles), the mixture was measured by the spectropolarimeter. The buffer used here contained 50 mmol/l Tris-HCl and 25 mmol/l NaCl at pH 7.4.

ratio in membrane: there was no β-sheet content when the cholesterol content was above 30 mol% (the contents of cholesterol of vesicles in the following text all refer to molar percentage), while 21.1% and 29% β-sheet content were found in 20% cholesterol containing PC vesicles and pure PC vesicles buffer respectively. Our previous work showed that the membrane insertion ability enhanced with the increase of cholesterol content, and the aggregation of Aβ could be greatly reduced when the cholesterol content was higher than 30% (Ji et al. 2002a). Therefore the relatively large change induced by cholesterol containing PC vesicles may mainly ascribed to the membrane insertion; and the elimination of β-sheet content presented by current study may partly account for the reduction of peptide aggregation.

In the process of CD measurement, we also detected the time course of DMPC vesicles with or without 33% cholesterol (data not shown). For 33% cholesterol containing DMPC vesicles, the β-sheet conformation of Aβ increased about 7% when Aβ interacted with cholesterol containing vesicles for 30 min, which was similar to the conformation after interacted with DMPC vesicles and maybe represented

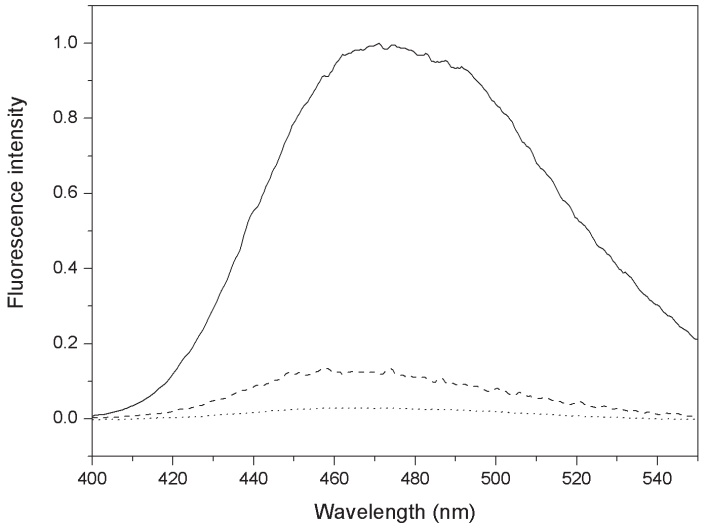

Figure 6. The exposure of hydrophobic sites of Aβ in present and absent of vesicles detected by ANS fluorescence. The ANS fluorescence of Aβ in aqueous solution (·····), in PC vesicles (——) and 33% cholesterol containing PC vesicles (– – –) was shown in the figure. The measurement were performed immediately after CD examination with the diluted samples. The concrete process of experiment was similar to Fig. 4.

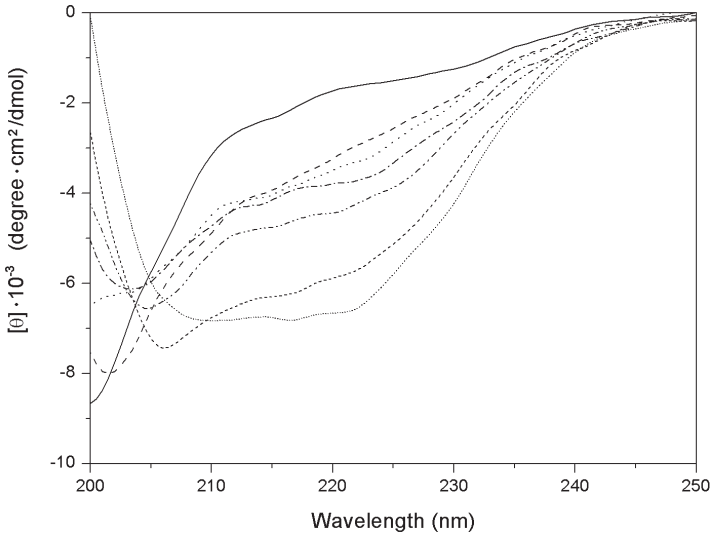

Figure 7. The effect of methanol-water system, simulating the environment of membrane, on the conformation of Aβ. Pure buffer (——), 20% (v/v) (– – –), 30% (·····), 40% (–·–·), 50% (––··––··), 60% (------) and 70% methanol containing buffer (·····) were used in experiments. The buffer used was neutral pH to simulate the neutral membrane system.

the conformation of Aβ on the surface of vesicles. Then the conformation changed mainly to α-helix after 60 min interaction and kept so until 90 min. But for DMPC vesicles, β-sheet would increase until the final conformation.

The change of hydrophobic exposure of Aβ in cholesterol containing PC system

The results of ANS detection for Aβ with vesicles were shown in Figure 6. The ANS fluorescence intensity of Aβ increased remarkably when incubated with PC vesicles, while only a small increase was detected when incubated with 33% cholesterol containing vesicles. This fact indicates more ANS molecules attach to the nonpolar region of Aβ in pure PC liposome solution. The corresponding time course of ANS experiments also were performed (data not shown). The results showed that the ANS fluorescence had a little increase at first and then a distinctly decrease after 60 min for cholesterol containing vesicles. But for the DMPC vesicles, the ANS fluorescence increased obviously along with the interaction time.

Since the ANS molecule would be in the vicinity of glycerol backbone of phospholipid without insertion into the hydrophobic region of membrane (Kachel et al. 1998), the increase of ANS fluorescence intensity would contribute to the exposure of hydrophobic sites of Aβ. Our previous work (Ji et al. 2002a) has proved that Aβ would insert into 33% cholesterol containing PC vesicles by its hydrophobic C-terminal. Thus most of the hydrophobic sites of Aβ were protected by the membrane containing 33% cholesterol, and consequently they could not lead to the ANS binding and the subsequent increase of fluorescence intensity. On the other hand, PC vesicles won't induce the membrane insertion of Aβ, which may result in the higher ANS fluorescence density.

The simulation of membrane surface by methanol-water mixtures

Åkerlöf (1932) reported that methanol contained buffer could be used to simulate the dielectric constant of membrane surface. Here we used this system to measure the conformation of Aβ in solutions, which could simulate the environment of the membrane. The results in Figure 7 showed that the α-helix content of Aβ conformation increased when more methanol was in the mixture, which was similar with the report of Terzi et al. (1997). According to the simulation by Yang's, the most similar conformation to that on the surface of PC vesicles is in 50% methanol mixture (10.8% α-helix and 30.5% β-sheet); the dielectric constant of this mixture is about 59, which is also similar to that of the membrane surface microenvironment (Tocanne and Teissie 1990).

Discussion

As the core protein of senile plaques, many studies were carried on Aβ, and parts of them were centralized in the conformation of Aβ, which may due to the relationship between the neurotoxicity and its conformation. Since the fibril formation and the membrane insertion are both involved with hydrophobic interactions and proved

to be important for the pathology of AD, the correlation of secondary structure of Aβ with the hydrophobic exposure under different conditions was investigated.

Our results showed that ion strength had little effect on both the secondary structure and the hydrophobic exposure of Aβ; while lower pH could induce more β-sheet conformation and higher hydrophobic fluorescence intensity. According to our work on monolayer measurement (Ji et al. 2000b), ion strength almost has no effect on the membrane insertion ability of Aβ, while lower pH could enhance the membrane insertion ability of Aβ. Since the membrane insertion is mainly a hydrophobic interaction, and the C-terminal (hydrophobic part) of Aβ was proved to be important for the membrane insertion (Song et al. 1999; Ji et al. 2002a) and fibril formation (Lansbury et al. 1995; McLaurin et al. 1998b), the hydrophobic exposure may partly account for above protein-membrane interaction behavior. Because different ion strength could not induce the conformational change, the hydrophobic exposure reasonably remained unchanged, which was further verified by ANS detection. This results in the inutility of ion strength on the membrane insertion ability of Aβ. On the other hand, Aβ has different conformation under different pH: more β-sheet conformation content is induced with the decrease of pH. This leads to the increase of hydrophobic exposure, and subsequently promotes the membrane insertion and fibril formation (proved *in vitro*, for example, McLaurin et al. 1998a) of Aβ.

The microenvironment of the membrane surface e.g. higher solute concentration than bulk concentration, and the protein-membrane interaction are the most important factors that affect the conformation of Aβ. It has been well documented that it is the membrane composition that regulate Aβ's behavior to a large extent (for example, Klunk et al. 1997; McLaurin and Chakrabarty 1997; McLaurin et al. 1998a,b). Our previous study indicated that the membrane insertion ability enhanced with the increase of cholesterol content, and when the content is higher than 30%, Aβ could insert into the physiological cholesterol containing PC bilayer (Ji et al. 2002a). In other word, Aβ could not penetrate PC vesicles with a cholesterol content less than 30%. Therefore the change in protein state with such vesicles from aqueous solution was mainly laid on the microenvironment near the membrane surface; and obviously the difference should not be very significant, which was also been verified by our results: PC vesicle solution could induce a little more α-helix and β-sheet conformation compared to that in aqueous solution. The simulation of membrane surface microenvironment using methanol mixture also gave consistent conclusion: although small, the membrane microenvironment did induce certain change in protein conformation. On the other hand, when incubated with cholesterol containing PC vesicles case became different: protein-membrane interaction turned to be the main factor, and thus induced a significant conformation change.

The cholesterol content in outer membrane of normal aged individuals would be about 30% (Ji et al. 2002a). The membrane insertion process may lead to the wrap of hydrophobic sites of Aβ (verified by the ANS detection, shown in Figure 6), and therefore decrease the possibility of the hydrophobic interaction between Aβ

molecules. Furthermore, the elimination of β-sheet content when incubated with 30% cholesterol containing PC vesicles presented by current study may benefit the reduction of peptide aggregation which was also proved by electron microscopy observations (Ji et al. 2002a). These two facts may partly account for the survival from AD in normal aged brain.

In the case for AD subjects, however, a significant difference is present: brain membranes isolated from them show obvious decrease in membrane cholesterol content (the ratio of cholesterol to phospholipids decreases about 30%) (Mason et al. 1992; Roth et al. 1995). Current results showed that more β-sheet would be induced when the cholesterol content in PC vesicles was less than 30%. In such cases, the membrane insertion ability also decreased (Ji et al. 2002a), which may account for the higher ANS fluorescence intensity of pure PC vesicles than that of cholesterol containing PC vesicles. Above facts could all promote the fibril formation, and thus may partly account for the aggregation of Aβ in AD brains.

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References

Åkerlöf G. (1932): Dielectric constants of some organic solvent-water mixtures at various temperatures. J. Am. Chem. Soc. **54**, 4125—4139

Ames B. N. (1966): Assay of inorganic phosphate, total phosphate phosphatases. Methods Enzymol. **8**, 115—118

Brown M. S., Goldstein J. L. (1997): The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell **89**, 331—340

Eckert G. P., Cairns N. J., Maras A., Gattaz W. F., Muller W. E. (2000): Cholesterol modulates the membrane-disordering effects of β-amyloid peptides in the hippocampus: specific changes in Alzheimer's disease. Dement. Geriatr. Cogni. Disord. **11**, 181—186

Esch F. S., Keim P. S., Beattie E. C., Blacher R. W., Culwell A. R., Oltesdorf T., McClure D., Word P. J. (1990): Cleavage of amyloid β peptide during constitutive processing of its precursor. Science (Washington, D. C.) **248**, 1122—1128

Haass C., Schlossmacher M. G., Hung A. Y., Vigo-Pelfrey C., Mellon A., Ostaszewski B. L., Lieberburg I., Koo E. H., Schenk D., Teplow D. B., Selkoe D. J. (1992): Amyloid β-peptide is produced by cultured cells during normal metabolism. Nature **359**, 322—325

Hardy J. (1997): Amyloid, the presenilins and Alzheimer's disease. Trends Neurosci. **20**, 154—159

Howland D. S., Trusko S. P., Savager M. J., Reaume A. G., Lang D. M., Hirsch J. D., Maeda N., Siman R., Greenberg B. D., Scott R. W., Flood D. G. (1998): Modulation of secreted β-amyloid precursor protein and amyloid β-peptide in brain by cholesterol. J. Biol. Chem. **273**, 16576—16582

Inouye H., Fraser P. E., Kirschner D. A. (1993): Structure of β-crystallite assemblies formed by Alzheimer β-amyloid protein analogues: analysis by X-ray diffraction. Biophys. J. **64**, 502—519

Ji S. R., Wu Y., Sui S. F. (2002a): Cholesterol is an important factor affecting the membrane insertion of amyloid peptide (Aβ1–40), which may potentially inhibit the fibril formation. J. Biol. Chem. **277**, 6273—6279

Ji S. R., Wu Y., Sui S. F. (2002b): The study of β-amyloid peptide (Aβ40) insertion into phospholipid membranes using monolayer technique. Biochemistry (Moscow) **67**, 1283—1288

Kachel K., Asuncion-Punzalan E., London E. (1998): The location of fluorescence probes with charged groups in model membranes. Biochim. Biophys. Acta **1374**, 63—76

Klunk W. E., Xu C. J., McClure R. J., Panchalingam K., Stanley J. A., Pettegrew J. W. (1997): Aggregation of β-amyloid peptide is promoted by membrane phospholipid metabolites elevated in Alzheimer's disease brain. J. Neurochem. **69**, 266—272

Lansbury P. T., Costa P. R., Griffiths J. M., Simon E. J., Auger M., Halverson K. J., Kocisko D. A., Hendsch Z. S., Ashburn T. T., Spencer R. G. S., Tider B., Griffin R. G. (1995): Structural model for the β-amyloid fibril based on interstrand alignment of an anti-parallel-sheet comprising a C-terminal peptide. Nat. Struct. Biol. **2**, 990—997

Martinez-Senac M. M., Villalaín J., Gómez-Fernández J. C. (1999): Structure of the Alzheimer β-amyloid peptide (25–35) and its interaction with negatively charged phospholipid vesicles. Eur. J. Biochem. **265**, 744—753

Mason R. P., Shoemaker W. J., Shajenko L., Chambers T. E., Herbette L. G. (1992): Evidence for changes in the Alzheimer's disease brain cortical membrane structure mediated by cholesterol. Neurobiol. Aging **13**, 413—419

Mason R. P., Estermyer J. D., Kelly J. F., Mason P. E. (1998): Alzheimer's disease amyloid β peptide 25–35 is localized in the membrane hydrocarbon core: X-ray diffraction analysis. Biochem. Biophys. Res. Commun. **222**, 78—82

Matsuzaki K., Horikiri C. (1999): Interactions of amyloid β-peptide (1–40) with ganglioside-containing membranes. Biochemistry **38**, 4137—4142

McLaurin J., Chakrabarty A. (1997): Characterization of the interactions of Alzheimer β-amyloid peptides with phospholipid membranes. Eur. J. Biochem. **245**, 355—363

McLaurin J., Franklin T., Chakrabartty A., Fraser P. E. (1998a): Phosphatidylinositol and inositol involvement in Alzheimer amyloid-β fibril growth and arrest. J. Mol. Biol. **276**, 183—194

McLaurin J., Franklin T., Fraser P. E., Chakrabarrty A. (1998b): Structural transitions associated with the interaction of Alzheimer β-amyloid peptides with gangliosides. J. Biol. Chem. **273**, 4506—4515

Mills J., Reiner P. B. (1999): Regulation of amyloid precursor protein cleavage. J. Neurochem. **72**, 443—460

Mori H., Takio K., Ogawara M., Selkoe D. J. (1992): Mass spectrometry of purified amyoid β protein on Alzheimer's disease. J. Biol. Chem. **267**, 17082—17086

Murphy M. P., Hickman L. J., Eckman C. B., Uljon S. N., Wang R., Golde T. E. (1999): γ-secretase, evidence for multiple proteolytic activities and influence of membrane positioning of substrate on generation of amyloid β peptides of varying length. J. Biol. Chem. **274**, 11914—11923

Racchi M., Baetta R., Salvietti N., Ianna P., Franceschini G., Paoletti R., Fumagalli R., Govoni S., Trabucchi M., Soma M. (1997): Secretory processing of amyloid precursor protein is inhibited by increase in cellular cholesterol content. Biochem. J. **322**, 893—898

Roher A. E., Lowenson J. D., Clarke S., Wood A. S., Cotter R. J., Gowing E., Ball M. J. (1993): β-amyloid-(1–42) is a major component of cerebrovascular amyloid deposits: implications for the pathology of Alzheimer disease. Proc. Natl. Acad. Sci. U.S.A. **90**, 10836—10840

Roth G. S., Joseph J. A., Mason R. P. (1995): Membrane alterations as causes of impaired signal transduction in Alzheimer's disease and aging. Trends Neurosci. **18**, 203—206

Selkoe D. J. (1997): Neuroscience- Alzheimer's disease: Genotypes, phenotype, and treatment. Science (Washington D. C.) **275**, 630—631

Shin R. W., Ogino K., Kondo A., Saido T. C., Trojanowski J. Q., Kitamoto T., Tateishi J. (1997): Amyloid beta-protein (Aβ) 1–40 but not Aβ 1–42 contributes to the experimental formation of Alzheimer disease amyloid fibrils in rat brain. J. Neurosci. **17**, 8187—8193

Song Y. W., Shim M. J., Kim S. W. (1999): Simulation study of behavior of β-amyloid peptide (βAP) on lipid membrane using cellular automata (CAs). J. Industr. Eng. Chem. **5**, 302—305

Stryer L. (1965): The interaction of a naphthalene dye with apomyoglobin and apohemoglobin: a fluorescent probe of non-polar binding sites. J. Mol. Biol. **13**, 482—495

Suzuki N., Cheung T. T., Cai X. D., Odaka A., Otvos L. Jr., Eckman C., Golde T. E., Younkin S. G. (1994): An increased percentage of long amyloid-β secreted by familial amyloid β protein-precursor (β-APP717)) mutants. Science (Washington D. C.) **264**, 1336—1340

Terzi E., Hölzemann G., Seelig J. (1997): Interaction of Alzheimer β-amyloid peptide (1–40) with lipid membranes. Biochemistry **36**, 14845—14852

Tocanne J. F., Teissie J. (1990): Ionization of phospholipids and phospholipid-supported interfacial lateral diffusion of protons in membrane model systems. Biochim. Biophys. Acta **1031**, 111—142

Waiter M. F., Mason P. E., Mason R. P. (1997): Alzheimer's disease amyloid β peptide 25–35 inhibits lipid peroxidation as a result of its membrane interactions. Biochem. Biophys. Res. Commun. **233**, 760—764

Xia X. F., Sui S. F. (2000): The membrane insertion of trichosanthin is membrane-surface-pH dependent. Biochem. J. **349**, 835—841

Yang J. T., Wu C. S., Martinez H. M. (1986): Calculation of protein conformation from circular dichroism. Methods Enzymol. **130**, 208—269

Yeagle P. L. (1985): Cholesterol and the cell membrane. Biochim. Biophys. Acta **822**, 267—287

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