The interaction of Aβ peptides with the lipid matrix of neuronal cell membranes plays an important role in the pathogenesis of Alzheimer's disease. By using EPR and CD spectroscopy, we found that in the presence of Cu²⁺ or Zn²⁺, pH, cholesterol, and the length of the peptide chain influenced the interaction of these peptides with lipid bilayers. In the presence of Zn²⁺, Aβ40 and Aβ42 both inserted into the bilayer over the pH range 5.5–7.5, whereas Aβ40 only penetrated the lipid bilayer in the presence of Cu²⁺ at pH 5.5–6.5; at higher pH there was a change in the Cu²⁺ coordination sphere that inhibited membrane insertion. In the absence of the metals, insertion of both peptides only occurred at pH < 5.5. Raising cholesterol to 0.2 mol fraction of the total lipid inhibited insertion of both peptides under all conditions investigated. Membrane insertion was accompanied by the formation of α-helical structures. The nature of these structures was the same irrespective of the conditions used, indicating a single low energy structure for Aβ in membranes. Peptides that did not insert into the membrane formed β-sheet structures on the surface of the lipid.

Alzheimer's disease (AD) is a neurodegenerative disorder affecting the memory and cognitive functions of the brain. A characteristic central nervous system histologic marker in patients with AD is accumulation of the 39–43-residue amyloid β-peptide (Aβ) in morphologically heterogeneous neuritic plaques and cerebrovascular deposits (1). Aβ is derived from a single membrane-spanning domain. Possible mechanisms of Aβ toxicity include formation of plasma membrane channels (2) and generation of H₂O₂ through Cu²⁺ oxidation (3) that may also lead to an increase in membrane permeability. The common change observed in cell membrane permeability is an increased intracellular calcium level (5, 6) that could occur either indirectly through Aβ modulating an existing Ca²⁺ channel or directly through cation-selective channels formed by Aβ.

The supramolecular structure of membrane-associated Aβ, either as an ion channel or a fusogen, is unknown, although Durell et al. (7) have developed theoretical models for the structure of ion channels formed by the membrane-bound Aβ40. Recently, Bhattacharjee et al. (8) and Lin et al. (9) used atomic force microscopy, laser confocal microscopy, and fluorescent calcium imaging to examine in real time the acute effects of fresh and globular Aβ40, Aβ42, and Aβ25–35 on cultured endothelial cells. Aβ peptides caused morphological changes within minutes after treatment and led to eventual cellular degeneration. Cellular morphological changes were most sensitive to Aβ42, being seen at nanomolar concentrations and accompanied by an elevated intracellular calcium level. Atomic force microscopy of Aβ42 reconstituted in a planar lipid bilayer showed multimeric channel-like structures. Biochemical analysis showed that the predominantly monomeric Aβ peptides in solution formed stable tetramers and hexamers after incorporation into lipid membranes. In our initial study using the EPR technique (10), we found that, in the presence of Cu²⁺ and Zn²⁺, Aβ42 formed allosterically ordered α-helical structures that penetrated negatively charged membranes. This paper extends that study to include the influence of pH, metal concentration, peptide length, and cholesterol on the conformation and incorporation of the peptide into the lipid bilayer. We show that penetration of the bilayer is closely related to conditions favorable to oligomerization of the peptide. The significance of this finding is that metals play an important part in the formation of amyloid plaques, the end point of oligomerization. Cherny et al. (11) have shown that administration of a lipophilic Cu²⁺/Zn²⁺ chelator (5-chloro-7-iodo-8-hydroxyquinoline) that crosses the blood-brain barrier attenuates plaque formation in APP2576 transgenic mice expressing human APP. Recently, the importance of Zn²⁺ in plaque formation has been emphasized by the finding that the age and female sex-related plaque formation characteristic of these mice was greatly reduced if they lacked zinc transporter 3, which is required for zinc transport into synaptic vesicles (12).
Materials and Methods—Peptides were obtained from AusPep (Melbourne, Australia) and from the W. M. Keck Laboratory, Yale University. The acidic phospholipid spin label 16NPS was synthesized according to Hubbell and McConnell (13). The water-soluble spin label TCC was obtained from Molecular Probes. All spin probes were checked for purity and to ensure that their number of spins/mole were >90% of theory (14). Cholesterol and synthetic POPC was purchased from Sigma, and POPS was purchased from Avanti Polar Lipids (Pelham, AL). LUV were prepared by the method described by Hope et al. (15). Peptides as a freeze-dried powder were added to the desired concentration to a suspension of LUV in buffer, and the mixture was vortexed under N2 for 10 min at 305 K in polypropylene tubes. One hundred-millimolar stock solutions of analytical reagent grade CuCl2 or ZnCl2 were prepared, and the desired amount was added to the LUV after addition of the peptide. 0.05 m of the chelator EGTA was added to all control solutions to counter the possible effects of any trace metals present. For copper EPR measurements, 99.99% pure 65Cu (Cambridge Isotopes) was used. Metal concentrations were measured by ICP-MS (model 700, Varian), and peptide concentrations were determined by quantitative amino acid analysis. Uptake of peptide by the LUV was estimated by density gradient centrifugation in Metrizamide™ (Nygaaard, Oslo, Norway)-Tris buffer (pH 7.0, 0.05 m) as described by Corbell et al. (16) and then measuring the extinction at 210 nm of the sub-phase. The protein concentration was estimated by using an ε<sub>210</sub> of 204 (17). It was found that 10–15% of the added peptide was not taken up by the LUV.

EPR Spectroscopy—Continuous wave X-band EPR spectra were obtained using a Bruker ECS106 spectrometer equipped with a temperature controller and flow-through liquid nitrogen cryostat. Cu<sup>2+</sup> spectra were collected at 110 K from samples contained in 4-mm inner diameter “Suprasil” quartz EPR tubes (Wilmsad). In order to eliminate the possibility that any line broadening observed in the spectra might be due to freezing-induced localized concentrations of sample, 10% glycerol was added to aqueous peptide buffer solutions. Labeled lipid samples (25 µl) were contained in 0.8-mm inner diameter quartz capillaries (Wilmad) and handled as described by Gordon et al. (18) to ensure reproducibility. Other procedures, including adding to the spin-labeled SUV a small amount of the water-soluble, non-membrane penetrant spin probe TCC to ensure x axis reproducibility, have been described previously (10, 19).

The analysis of EPR spectra of peptide/lipid mixtures was carried out using the spectral subtraction and addition methods described by Marsh (20). As a check on the validity of these procedures as applied to our system where an MRLC was observed, the lipid spin label spectra were simulated using the modified Bloch equations as described by Davoust and Devaux (21). Their model I was used in the simulations where the unique director orientation in the fluid component, corresponding to the nitroxide axes being aligned along the membrane normal in the MRLC, was assumed to be preserved on exchange.

RESULTS

Effect of pH and Peptide Length on Interaction of Aβ Peptides with LUV—Fig. 1 shows the EPR spectra recorded at 305 K of 16NPS in POPS/POPC (1/1) LUV and the same LUV containing Aβ42 before and after the addition of Cu<sup>2+</sup> (0.3/1 m peptide) at pH 7.5, 7.0, 6.5, 6.0, and 5.5. The sharp lines due to the TCC marker and the curved base line due to the broad Cu<sup>2+</sup> line have been subtracted as described previously (10). It can be seen that adding Cu<sup>2+</sup> to the vesicles at all pH values caused the appearance of the MRLC (marked with an arrow) that is characteristic of annular phospholipid surrounding a rigid peptide inserted into the bilayer (20). At pH 5.5 the spectrum of the non-Cu<sup>2+</sup>-treated Aβ-containing LUV clearly shows the

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**Fig. 1.** EPR spectra of 16NPS in POPS/POPC LUV over the pH range 5.5–7.5 (A), after the addition of Aβ42 (1 m peptide/30 m lipid) (B), after the addition of 0.3 m Cu<sup>2+</sup>/1 m peptide to the samples in B (C), and difference spectra obtained by subtracting the spectra in A from the corresponding spectra in C (D). Spectra were recorded at 305 K. Instrument conditions are as follows: microwave frequency 9.237 MHz, modulation frequency 100 KHz, modulation amplitude 3.2 G, microwave power 6 milliwatts, sweep time 400 s, and time constant 0.5 s. Scan width was 100 G and mid-field 3,300 G.
MRLC, at a slightly lower intensity than in the Cu\textsuperscript{2+}-treated sample at the same pH. The control spectra (set A) were subtracted from those of the Cu\textsuperscript{2+}-treated LUV and from the pH 5.5 peptide-containing LUV to give the difference spectra (set D). These experiments were repeated over the same range of pH with Zn\textsuperscript{2+} (4/1 m peptide) and Aj424 and with Cu\textsuperscript{2+} and Zn\textsuperscript{2+} at the respective 0.3 and 4.0 molar ratios with Aj40. No MRLC was found with rat Aβ40 with both metals over the whole pH range (data not shown). By performing the subtraction with spectra of control LUV made at 286 K, as done previously with both Aβ40 (10) and a viral peptide (19), it was shown that the peptides did not perturb the bulk lipid, as suggested by McIntyre et al. (22). All of the spectra could be simulated over the range of peptide/lipid mixtures used, as described by Davoust and Devaux (21), further discounting long range perturbation effects on the bulk lipid by the peptide at high peptide/lipid. The on- and off-rate constants were estimated from the simulation; the former were in the range 6–5.5 \times 10^6 (s\textsuperscript{-1}), and the latter were 5.5–5 \times 10^5 (s\textsuperscript{-1}). These values are similar to those published for other lipid/protein systems (23).

The proportion of MRLC to total lipid was calculated by double-integrating the five sets of difference spectra thus obtained and expressing the number of spins as a percentage of the number of spins in each experimental spectrum. The values are given in Table I. It can be seen that, with Aβ42, decreasing pH with Cu\textsuperscript{2+} and Zn\textsuperscript{2+} leads to an increase in the MRLC. It is notable that there is a significant MRLC at pH 5.5 in the absence of either metal. With Aβ40, however, there is a marked difference between the behavior of Cu\textsuperscript{2+} with pH in that there is negligible MRLC at pH 7.0 and 7.5 with the Cu\textsuperscript{2+}, although with the Zn\textsuperscript{2+} the proportion of MRLC at all pH values is similar to that found with Zn\textsuperscript{2+} and Aj424. Adding Zn\textsuperscript{2+} to the pH 7.0 and 7.5 Aβ40/Cu\textsuperscript{2+} samples (2 m Zn\textsuperscript{2+}/0.3 m Cu\textsuperscript{2+}) resulted in the appearance of the MRLC. At the lipid/peptide ratio employed (30/1), the proportion of MRLC corresponded approximately to four lipid molecules/peptide. This value was previously observed to remain constant for Aβ42 (10) over a range of lipid/peptide ratios, and it can be satisfied on structural grounds by postulating that the basic membrane penetrant unit is a α-helical hexamer surrounded by 24 lipids (10).

We have shown previously (10) that the appearance of the MRLC in the Aj42 negatively charged LUV system was associated with an increase in α-helicity of the peptide. The % α-helix (bold type) and β-structure (italics) are shown alongside the MRLC data in Table I. It can be seen that wherever significant MRLC is present, the peptide showed increased α-helicity. Wherever the peptide does not show a MRLC, there is a preponderance of β-structure.

**Effect of pH on the Coordination Sphere of Cu\textsuperscript{2+} in Both Peptides in the LUV—EPR spectra taken at 110 K of \textsuperscript{65}Cu\textsuperscript{2+}-Aβ28 in aqueous buffer over the pH range 5.5–7.5 (Cu\textsuperscript{2+}/peptide 0.3/1 m).** B, EPR spectra taken at 110 K of Cu\textsuperscript{2+}-Aβ28 in aqueous buffer over the pH range 5.0–8.0 (Cu\textsuperscript{2+}/peptide 0.7/1 m). Instrument settings are as follows: frequency A, 9.7660 GHz; frequency B, 9.7655, modulation frequency 100 KHz, modulation amplitude 1.011 G, microwave power 2 milliwatts, sweep time 83.388 s, time constant 0.240 ms. Spectra averaged over 36 sweeps. Planar copper. However, the notable feature of the series is a decrease in g∥ (marked with arrow in Fig. 2A) between pH 5.5 and 6.0 and a further decrease between pH 7.0 and 7.5 indicating a change in the \textsuperscript{65}Cu\textsuperscript{2+} coordination sphere over these two pH ranges. The spectral parameters are given in Table II. As observed previously (10) for Aβ42, spectra for \textsuperscript{65}Cu\textsuperscript{2+}-Aβ40 in 16NPS-LUV showed no signs of line broadening due to dipolar-induced spin lattice relaxation by the nitroxide-free radical (24), indicating that the Cu\textsuperscript{2+}-binding site of the peptide did not penetrate the bilayer and come into the proximity of the nitroxide spin label. However, Cu\textsuperscript{2+} molar ratio-dependent line broadening due to Heisenberg exchange, the result of Cu\textsuperscript{2+} atoms being close in space, had been observed previously in Aβ complexes (10). Those spectra had been recorded of samples in pH 6.9 buffer. Fig. 2B shows the effect of a range of

| pH  | No metal | Cu\textsuperscript{2+} | Zn\textsuperscript{2+} | No metal | Cu\textsuperscript{2+} | Zn\textsuperscript{2+} |
|-----|----------|-----------------|---------------|----------|-----------------|---------------|
| 7.5 | 0 (65, 13) | 10 (18, 55) | 9 (17, 53) | 0 (71, 4) | 2 (63, 18) | 8 (41, 61) |
| 7.0 | 0 (67, 11) | 10 (13, 57) | 10 (17, 56) | 0 (70, 4) | 3 (67, 21) | 10 (16, 65) |
| 6.5 | 0 (65, 10) | 11 (15, 56) | 11 (15, 58) | 0 (72, 3) | 9 (22, 53) | 11 (15, 58) |
| 6.0 | 2 (47, 26) | 12 (16, 58) | 11 (15, 48) | 0 (69, 7) | 11 (17, 63) | 11 (15, 61) |
| 5.5 | 8 (18, 50) | 12 (13, 57) | 13 (15, 55) | 7 (19, 51) | 12 (16, 58) | 13 (10, 64) |

**Fig. 2.** A, EPR spectra taken at 110 K of Cu\textsuperscript{2+}-Aβ28 in aqueous buffer over the pH range 5.5–7.5 (Cu\textsuperscript{2+}/peptide 0.3/1 m). B, EPR spectra taken at 110 K of Cu\textsuperscript{2+}-Aβ28 in aqueous buffer over the pH range 5.0–8.0 (Cu\textsuperscript{2+}/peptide 0.7/1 m). Instrument settings are as follows: frequency A, 9.7660 GHz; frequency B, 9.7655, modulation frequency 100 KHz, modulation amplitude 1.011 G, microwave power 2 milliwatts, sweep time 83.388 s, time constant 0.240 ms. Spectra averaged over 36 sweeps. Planar copper. However, the notable feature of the series is a decrease in g∥ (marked with arrow in Fig. 2A) between pH 5.5 and 6.0 and a further decrease between pH 7.0 and 7.5 indicating a change in the \textsuperscript{65}Cu\textsuperscript{2+} coordination sphere over these two pH ranges. The spectral parameters are given in Table II. As observed previously (10) for Aβ42, spectra for \textsuperscript{65}Cu\textsuperscript{2+}-Aβ40 in 16NPS-LUV showed no signs of line broadening due to dipolar-induced spin lattice relaxation by the nitroxide-free radical (24), indicating that the Cu\textsuperscript{2+}-binding site of the peptide did not penetrate the bilayer and come into the proximity of the nitroxide spin label. However, Cu\textsuperscript{2+} molar ratio-dependent line broadening due to Heisenberg exchange, the result of Cu\textsuperscript{2+} atoms being close in space, had been observed previously in Aβ complexes (10). Those spectra had been recorded of samples in pH 6.9 buffer. Fig. 2B shows the effect of a range of
pH on this line broadening. It can be seen that, at the 0.7 Cu$^{2+}$-molar ratio used, line broadening was present from pH 6 to 7, much reduced at pH 7.5, and eliminated at pH 8. The broadened spectrum at pH 5, which is markedly different to that seen at pH 6–7, was due to the presence of unbound Cu$^{2+}$, indicating a weakening of the strong binding to the metal observed at the higher pH values, and corresponding to the protonation of the imidazole side chain of the histidine residues. There were no peaks at $g > 4$ (not shown in Fig. 2B), indicating the absence of axial Cu$^{2+}$ dimers, nor was there any marked reduction of signal intensity, diagnostic of the formation of EPR "silent" dicopper (25).

From Fig. 3 it can be seen that the increase in the amount of MRLC at pH 6.0 with both peptides reaches a maximum at a Cu$^{2+}$-concentration of 1 mol/mol, after which it remains constant. The effect of Zn$^{2+}$ follows a similar pattern, albeit at higher concentrations, requiring almost 3 eq in keeping with the lower affinity of A$\beta$ for Zn$^{2+}$ (26).

Effect of Cholesterol on the MRLC—In the light of numerous reports on the significance of cholesterol on A$\beta$–membrane interactions in both biological membranes and model systems (27–29), the effect of adding cholesterol to the LUV on MRLC formation was investigated. The results are summarized in Table III where it can be seen that at each pH value and metal where the component was observed in the absence of cholesterol it was reduced to zero when cholesterol represented 0.2 mol fraction of the total lipid, i.e. cholesterol reduced the stability of the membrane-penetrant form of the peptide.

The Effect of Adding Cu$^{2+}$ to Zn$^{2+}$–A$\beta$40 in the Spin-labeled LUV—Because it had been observed that the appearance of MRLC in the spin-labeled LUV after the addition of Zn$^{2+}$–A$\beta$40 was independent of pH, and it was pH-dependent after the addition of Cu$^{2+}$–A$\beta$40 (Table I), the effect of adding Cu$^{2+}$ to Zn$^{2+}$–A$\beta$40 LUV was explored. It can be seen (Fig. 4) that at pH 7.0 the addition of Cu$^{2+}$ markedly reduced the percent MRLC until none was detectable at 1 mol Cu$^{2+}$/mol of peptide. This result indicates that Cu$^{2+}$ competes with Zn$^{2+}$ for at least one of the His residues of A$\beta$40, but with a coordination sphere such that the stability of the complex is too low to promote membrane insertion.

**DISCUSSION**

**pH, Metal-induced Membrane Penetration**—The addition of metal ion and pH are two conditions that govern the formation of the MRLC indicating peptide penetration of the hydrocarbon core of the membrane by A$\beta$ peptides in LUV (Fig. 1), and these conditions closely parallel those governing A$\beta$ oligomerization (30). In the absence of metals and in the presence of the chelating agent EGTA, both A$\beta$40 and A$\beta$42 penetrate the membrane at pH 5.5 but not at higher pH values. Both peptides aggregate in the absence of either metal at this pH (30). In the presence of Zn$^{2+}$ both peptides penetrated the membrane over the entire range from pH 5.5 to 7.5. Zn$^{2+}$ aggregates A$\beta$ across this pH range (31). A$\beta$40 in the presence of Cu$^{2+}$ only penetrated the membrane at pH 6.5 and below, pH values at which it has been reported to oligomerize (32). On the other hand, A$\beta$42 in the presence of Cu$^{2+}$ penetrates and is oligomerized in solution at all pH values. It should be noted that rat A$\beta$40, which has a 2N2O coordination (10) and much-reduced metal-induced oligomerization (33), did not produce an MRLC with either metal at any pH. The significance of this result is that the selectivity of human over rat A$\beta$40 is one of the key metal-differentiating effects that argues strongly for metals being germane to the pathophysiology of AD. The induction of MRLC by copper-bound A$\beta$42 was saturated at 1 eq (Fig. 3); this parallels our previous observation that the cooperative binding of Cu$^{2+}$ to A$\beta$ was also saturated at 1 eq (10).

Our results showed that membrane penetration was associated with an increase in $\alpha$-helicity as determined by CD spectroscopy, whereas the CD spectra of peptide associated with liposomes showing no evidence of MRLC indicated predominantly $\beta$-structure (Table I). These results indicated that peptide association with the hydrocarbon region of the lipid bilayer facilitated $\alpha$-helix formation. Previously published CD studies of the interaction between the A$\beta$ peptides and lipid vesicles of varying composition have suggested that the peptides associated with the lipids are in the $\beta$-strand conformation (34–36). Most of these studies were limited to A$\beta$25–35, a peptide that is too short to be able to span the lipid bilayer in an $\alpha$-helical conformation. On the other hand, NMR structural studies in SDS micelle systems show that residues 15–36 are $\alpha$-helical with a kink in the 25–27 region (37, 38).

Calculation of the annular lipid/peptide ratio allows an estimate of the size of the molecule/complex penetrating the membrane. For all the spectra exhibiting MLRC the same value of 4–4.5/1 was obtained, consistent with an $\alpha$-helical hexameric peptide structure inserted into the bilayer in line with our previous results (10). The constancy of this ratio over a range of peptide/lipid suggested that formation of multimers occurs at relatively low value of the latter and that their size remains constant with increasing proportion of peptide in the bilayer. Factors influencing the interpretation of the stoichiometry of lipid-protein interactions determined by EPR have been discussed in detail by Marsh and Horváth (39).

It has been postulated that the ion channels that have been observed in various membrane systems could consist of either strands arranged in a $\beta$-barrel conformation or as an $\alpha$-helical assemblage, probably a tetramer or hexamer (see Refs. 7–10 and the data presented here). It has been shown by Horváth et al. (23) and Wolls et al. (40), however, that the motion of the MRLC observed with membrane penetrant segments of known $\beta$-barrel structure is much more restricted than with $\alpha$-helical segments. This is because of a slower on-off rate for the lipids in the former case. The on-off rates calculated by simulation in our case are close to those observed for other $\alpha$-helical systems (20).
**Aβ-Lipid Interactions**

**TABLE III**

| Cholesterol | Aβ40  |  |  |  |  | Aβ42  |  |  |  |  |
|-------------|-------|---|---|---|---|-------|---|---|---|---|
|             | 0.00  | 0.05 | 0.10 | 0.15 | 0.20 | 0.00  | 0.05 | 0.10 | 0.15 | 0.20 |
| Cu²⁺        | 10    | 8   | 7   | 5   | 2   | 11    | 9   | 6   | 5   | 0   |
| Zn²⁺        | 8     | 8   | 7   | 5   | 1   | 10    | 7   | 5   | 2   | 1   |

Fig. 4. The effect of adding Cu²⁺ to Zn²⁺-Aβ40 in spin-labeled LUV at pH 7.0 on the percentage of MRLC. Lipid/peptide, 1/30, (molar), metal/peptide, 4/1 (mol/mol), temperature 294 K.

Adding metal ions or lowering the pH are two different methods of aggregating Aβ. In the absence of lipid, metal ions induce amorphous aggregates of Aβ, and these oligomers are different from those formed in the pH-induced oligomerization of Aβ. These have a fibrillar β-sheet structure as shown by Congo Red birefringence (41). In lipid systems, however, both methods of Aβ oligomerization give the same membrane-penetrating α-helical structure, suggesting a well-defined low energy structure for Aβ in membranes. The observation of an α-helical hexamer is consistent with the results of the atomic force microscopy study by Lin et al. (9) that showed that Aβ peptides were able to form hexameric channel-like structures. The greater stability of Aβ42 in the lipid is also consistent with the observation by Bhatia et al. (8) showing a higher tendency by Aβ42 to form channel-like structures and a greater ability to disrupt cellular Cu²⁺ regulation than Aβ40. The two extra hydrophobic residues at the C terminus of Aβ42 would result in this peptide having a higher preference for the hydrophobic core of the lipid bilayer. The effect of the extra two hydrophobic residues is amplified in the hexameric unit, as there are 12 extra hydrophobic residues per unit for Aβ42 versus Aβ40.

We have shown that Zn²⁺ will induce Aβ penetration of the lipid with the formation of structures that are similar to the channels proposed by Lin et al. (9). However, their results showed that Zn²⁺ and an antibody directed against the N terminus had an inhibitory effect on Aβ channel activity (9). Although our results show that metal ions can induce oligomerization and membrane penetration, the metal-binding site itself is outside the lipid bilayer (10). The antagonistic action of Zn²⁺ may be due to its ability to extensively cross-link the three histidine residues of Aβ to form a cap over the channel, physically blocking it.

**pH-induced Differences between Aβ40 and Aβ42**—There is a significant reduction in Cu²⁺-facilitated membrane insertion of Aβ40 at pH values of 7.0 and above, contrasting with Aβ42 that showed consistent membrane insertion across the pH range investigated. Our present and earlier EPR data (10) indicated that Cu²⁺ formed type 2 square planar complexes with Aβ peptides. The spectra were independent of the peptide chain length and the presence or absence of lipid, but were influenced by pH. For type 2 complexes the g₁ and Aₐ values correlated with the types of equatorially coordinated atoms. The decrease in g₁ (Fig. 2A and Table II) between pH 6.0 and 5.5 and a further decrease between pH 7.0 and 7.5 indicated a change in the Cu²⁺ coordination sphere over these two pH ranges. This is further illustrated in Fig. 2B where the Cu²⁺ molar fraction-dependent line broadening attributed to Heisenberg exchange effects (10) diminishes with increasing pH, suggesting a change in the coordination sphere such that the Cu²⁺ atoms are no longer close in space. This would be consistent with reduction in the extent of histidine bridging between Cu²⁺ centers, although this was not eliminated until pH 8. Such a change in the coordination mode reflects a change in the Cu²⁺ from a coordination sphere that promotes peptide oligomerization such as with a histidine residue bridging copper atoms (a coordination sphere that resembles the active site of superoxide dismutase) to a mode that does not. Miura et al. (42) and Suzuki et al. (43) have shown by using Raman spectroscopy that Cu²⁺ binds to the Nₑ of the three His residues of Aβ at pH 5.8, whereas at pH 6.6 and 7.4 it could bind to the Nᵣ and an as yet unidentified backbone amide nitrogen. Zn²⁺, on the other hand, binds to the Nᵣ over the pH range 5.8–7.4. A change in the coordination mode of the Cu²⁺ bound to Aβ at higher pH that also reduces the oligomerization of the peptides would explain the observed reduction of copper-induced MRLC by Aβ40 at these pH values. These observations are consistent with the reduced aggregation of Aβ by copper at higher pH (32). The extra hydrophobic stabilization of Aβ42 versus Aβ40 is able to shift the pH-induced change in equilibrium between the different coordination modes of Cu²⁺ back to the form that favors membrane penetration and peptide oligomerization; this is a form that resembles the active site of superoxide dismutase as we have shown previously (10). Zn, which does not undergo a pH-induced change in metal coordination, induces peptide insertion into the membrane with both Aβ40 and Aβ42 over the pH range investigated. However, from the data shown in Fig. 4 it appears that the 1 eq of copper at pH 7.0 will displace the zinc and inhibit Aβ40 insertion into the membrane. Competition between Cu²⁺ and Zn²⁺ at this pH for binding to at least one of the histidine residues would explain these results. The different Cu²⁺ coordination modes and, hence, lipid interactions may explain the increased toxicity normally associated with Aβ42 because this peptide is more stable in the lipid environment. **The Effect of Cholesterol on Membrane Penetration**—Cholesterol is a major component of mammalian cell membranes and is known to affect the dynamics and thickness of the hydrocarbon region of the membrane. It has been widely reported to modulate Aβ-membrane interactions, and there is strong circumstantial evidence that it has a role to play in AD (27–29). Increased membrane cholesterol reduces the membrane disordering effects of Aβ, as shown by fluorescence polarization techniques, and inhibits its effects on Ca²⁺ signaling (35). Our results show that enhanced membrane cholesterol has the marked effect of decreasing MRLC formation by Aβ. Penetration of the membrane by the peptides would depend on a balance between the rigidifying effects of cholesterol and the possible better match of the hydrophobic region of the peptide by the increased bilayer thickness. Because the α-helical hydrophobic regions of Aβ peptides span 22–25 residues (37), close to the 20–23-residue width of the unexpanded bilayer, it is likely that the rigidifying effects will predominate, resulting in peptide exclusion. Such exclusion in the presence of chole-
terol would explain the inhibitory effects of the latter on Ca\(^{2+}\) signaling, because Aβ-induced channels would be destabilized in lipid rich in cholesterol.

Overall, the data suggest the location and stability of Aβ peptides within the membrane is delicately balanced, and as such, small changes in pH and lipid composition would be able to affect the stability of the Aβ-lipid interactions. Because neuronal cells are in a dynamic environment, with respect to pH and lipid composition that varies with different sub-cellular membrane fractions, it is not surprising that other workers (44) have found wide variations in the effects of Aβ on membrane order and fluidity in such fractions. A challenge for the future will be to define the links between the biophysical observations made in these diverse systems with the pathophysiology of AD.

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