Aβ Metallobiology and the Development of Novel Metal-Protein Attenuating Compounds (MPACs) for Alzheimer's Disease

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Abstract: Over a decade of studies have pointed to metal mediated neural oxidative damage as an attractive target for the treatment of Alzheimer's disease. Because of the nature of the blood brain barrier, systemic depletion of the metals, copper, zinc and possibly iron, is not a viable approach. However preliminary studies with CQ, a blood brain barrier penetrating chelating agent, are showing promise. CQ probably works by combining with the metal centres, primarily copper and zinc complexes of Aβ, in the neuropil. This review discusses some of the background that resulted in CQ becoming a lead compound and how we might advance our understanding of its action

METALLOPROTEINS AND OXIDATION DAMAGE IN ALZHEIMER'S DISEASE

Increasing evidence emphasises the importance of metals in neurobiology. For example, copper-binding proteins in the central nervous system may possess oxidant or anti-oxidant properties, possibly affecting neuronal function or triggering neurodegeneration. Among the copper-binding proteins related to neurodegenerative disease is the amyloid precursor protein (APP) of Alzheimer's disease (AD) that has two copper-binding sites APP135-156, and near its N-terminus, APP1. APP is a highly conserved and widely expressed integral membrane protein with a single membrane-spanning domain. The amyloid β peptides (Aβ) are 39–43 residue polypeptides derived from proteolytic cleavage of APP, by the combined action of two proteases, BACE and γ-secretase. A characteristic central nervous system histological marker in AD patients is accumulation of morphologically heterogeneous neuritic plaques and cerebrovascular deposits of Aβ [1]. Both the APP135 – 156 and Aß have been shown to have copper reducing activity with concomitant production of reactive oxygen species (ROS) [2, 3].

It has been long-established that oxidative damage to many classes of biological molecule, including sugars, lipids, proteins and nucleic acids, is increased in AD [4-6]. Cu^{2+} and Fe^{3+} interact with Aβ to make it toxic in cell culture. *In vitro* Aβ catalyses H_2O_2 generation through the reduction of Cu^{2+} and Fe^{3+}, using O_2 and biological reducing agents, such as cholesterol, vitamin C and catecholamines, as substrates [7-10]. Consistent with these biochemical properties being responsible for disease, the neurotoxicity of Aβ in culture is mediated by the Aβ:Cu^{2+} (or Aβ:Fe^{3+}) forming H_2O_2 [8, 11].

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Aβ generation alone was once believed to engender toxicity. However, we found that Aβ was not toxic in the absence of Cu^{2+} or Fe^{3+} [3]. Although there have been reports of toxic fibrillar and toxic soluble oligomeric species of Aß, those studies have not yet excluded the possibility that the toxicity of the modified Aß species is dependent upon recruiting Cu^{2+} or Fe^{3+} from the culture medium. H_2O_2, being freely permeable across all tissue boundaries, unless scavenged by defences such as catalase and glutathione peroxidase, will react with Fe^{2+} and Cu^{+} to generate OH• radicals by the Fenton reaction [10] that, in various cellular compartments, generates the lipid peroxidation adducts, protein carbonyl modifications, and nucleic acid adducts such as 8-OH guanosine that are typical of AD neuropathology [4, 6, 12] and precede Aβ deposition [13, 14]. Metal-centered ROS generation reactions of this kind have also been reported to potentially mediate the neurotoxicity of PrP in transmissible spongioform encephalopathies and alpha-synuclein in Parkinson's disease [10, 15].

METAL MEDIATED OXIDATIVE DAMAGE AS A THERAPEUTIC TARGET

The oxidative damage hypothesis of AD gave rise to a great deal of discussion about the part that various types of antioxidants and free radical scavengers might play in preventing or delaying the onset of the disease. This became part of the wider interest in the role of such compounds in promoting general health. Unfortunately, there is little hard evidence of the beneficial effect of their consumption on Alzheimer's or any other neurodegenerative disease. Neither has any credible therapy been based on an antioxidant or free radical scavenger. On the other hand, experimental *in vitro* studies had shown that it was possible by sequestering the metal ion with a suitable chelator to block the production of ROS by copper complexed Aβ peptides. [16-18]. These findings suggested that it might be possible to develop a therapy based on metal complexation. Targeting a metal-

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binding site on a protein is not novel, and is quite different to chelation therapy, which aims to lower toxic metal burdens by sequestration. Examples of agents directed at metal sites are disulfiram, which chelates the zinc-catalytic site of alcohol dehydrogenase, blocking its activity [19], and the non-steroidal anti-inflammatory drugs, aspirin, diflunisal, ibuprofen, naproxen sodium, Indomethacin and D-penicillamine, which block the heme-iron catalytic site in the cyclooxygenase/arachidonic acid pathway [20]. An initial attempt to treat AD with the chelator desferrioxamine (DFO) showed a reduction in the rate of progression of the dementia [21], but this approach appears not to have been followed up. The reasons were probably that DFO requires twice-daily intramuscular injections and is a broad-spectrum chelator with a high affinity for iron, copper, zinc and aluminium that can cause systemic metal depletion. The affinity for aluminium was the rationale for the trial, since it was believed at the time that this metal played a role in producing the amyloid deposits and tau protein tangles in the brain, characteristic of the disease.

Studies of the action of a range of metal chelators on post-mortem AD-affected brain tissue found that the dissolution of AβP in amyloid plaques was correlated with the release of Cu and Zn, but not Fe [22]. Recently, Dong *et al*. [23] have shown with the aid of Raman microscopy that treatment of amyloid plaques with the chelator tetraethyldiamine tetraacetate leads to a loosening of their characteristic β−structure owing to a reversal of Cu^{2+} binding by the histidine residues of the plaques' constituent Aβ peptides. Consideration of the bioavailability of the chelators led to *in vivo* trials of clioquinol (5-chloro-7-iodo-8-hydroxyquinoline, CQ), (Fig. **1**). Oral treatment of human APP-expressing Tg2576 transgenic mice for 9 weeks with CQ caused a 49% decrease in brain Aβ deposition (-375 μg/g wet weight, p = 0.0001) in a blinded study [24]. Neurotoxicity was absent,

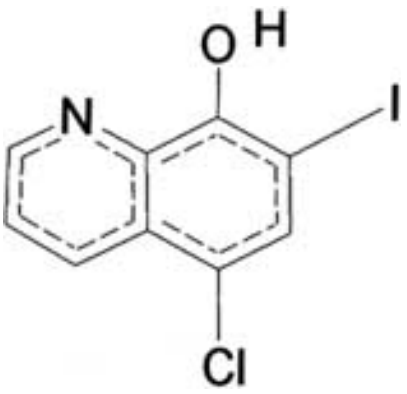

Fig. (1). Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline).

and general health and body weights were significantly more stable in the treated animals, which were conspicuously improved after only 16 days of treatment. Compared with the results of a study of an anti-Aβ vaccine in the PDAPP transgenic mouse [25], the inhibition of the absolute amount of cerebral Aβ deposition by CQ was more extensive, and occurred more rapidly. CQ treatment, therefore, appears like the vaccine therapy to be a potent inhibitor of Aβ accumulation. Treatment of the Tg2576 mice with the a hydrophilic copper chelator, tetraethyl triamine acetic acid (TETA) that could not cross the blood brain barrier did not inhibit amyloid deposition [24], indicating that systemic metal depletion (eg "chelation therapy") is not likely to be a useful therapeutic approach for AD. CQ has now proceeded into phase I [25] and phase II [27] clinical trials in AD patients.

These early findings with CQ have been treated with cautious optimism in some quarters because of the reputation that the compound has of being a neurotoxin itself. Thirty years ago, CQ was removed from the market as an antibiotic after its use was linked to some 10, 000 cases of subacute myelo-optic neuropathy (SMON), mostly in Japan [28], although it is still used in topical antibiotic preparations. SMON symptoms and distal axonopathy could be reproduced by giving high doses of CQ to dogs and cats [29], and more recently [30] it has been suggested that CQ zinc chelates were the neurotoxin involved in SMON. It has also been suggested that the high incidence of the disease in Japan might have been a consequence of that population's impaired vitamin B12 status after WWII [31]. In the Phase II trial the vitamin was coadminstered with CQ and no serious adverse effects were observed [27]. Another concern had been that dissolution of the plaques could cause a rise in soluble Aβ, which has been shown to be more neurotoxic than the insoluble form [32]. Again this concern has not been supported either by the transgenic mouse studies or in the clinical trial. Nevertheless, until its mechanism of action and its targets are thoroughly understood, there will be some concerns about long-term side effects with sustained maintenance or prevention therapy with CQ.

HOW DOES CQ WORK?

CQ is hydrophobic, unlike most chelators such as TETA and penicillamine, and readily crosses the blood brain barrier. It is uncertain how it is carried in the blood stream, but its low aqueous solubility may account for its lack of systemic metal depletion. It has been considered that CQ could work in the brain by facilitating dissolution of zinc-mediated Aβ plaques [33] as well as inhibiting Cu^{2+} or Fe^{3+}-mediated H_2O_2 production catalysed by Aβ [32]. Zn^{2+} plays a complex role in the pathophysiology of Aβ. It precipitates Aβ to form amyloid plaques [34] and, since the ion suppresses H_2O_2 production by Aβ, it has been suggested that plaque formation represents a defence where Zn^{2+} acts to sequester the abnormal activity of Aβ [35]. This might explain why H_2O_2-mediated oxidative damage in the neuropil is inversely correlated to plaque load. However, there is some recent evidence that the Aβ in plaque is insufficiently loaded with Zn^{2+} to completely abolish this adverse catalytic activity [3] and, therefore, Zn^{2+}-induced plaque formation might not be fully effective in preventing abnormal H_2O_2 production by Aβ. H_2O_2 also inhibits LRP-mediated clearance mechanisms [36]. The inhibition of metal-mediated H_2O_2 production from Aβ by CQ could facilitate Aβ clearance, with the resolubilized Aβ being removed into the blood, as in Aβ-immunized transgenic mice [37], or degraded by intracellular uptake and hydrolysis. CQ is as effective as high affinity chelators in blocking the production of H_2O_2 by Aβ *in vitro*, in preventing precipita-

tion of synthetic Aβ by Zn^{2+} and Cu^{2+}, and in extracting Aβ from post-mortem AD brain specimens [22]. Although Cu^{2+} and Fe^{3+} are also enriched in amyloid plaques (Zn^{2+}= 1055 μM, Cu^{2+}= 390 μM, Fe^{3+}= 940 μM), compared to normal age-matched neuropil (Zn^{2+}= 350 μM, Cu^{2+}= 70 μM, Fe^{3+}= 340 μM) [35], Aβ precipitation by Cu^{2+} and Fe^{3+} is far less than that induced by Zn^{2+} at pH 7.4 [33, 34], and when Aβ is precipitated by synaptic zinc, it can co-precipitate with Cu^{2+}, a possibility supported by the observation of selective Cu^{2+} and Zn^{2+} binding sites on Aβ [38].

The iron in amyloid plaque occurs predominantly in neuritic processes, probably complexed with ferritin [39], and may not directly interact with plaque Aβ. Unlike Cu and Zn, Fe does not copurify with Aβ from post-mortem AD brain tissue [3]. Also, in studies of various metal chelators solubilizing AβP from post-mortem AD-affected brain tissue, the dissolution of precipitated Aβ was correlated with the release of Cu and Zn, but not Fe [3]. Therefore, the dual Zn^{2+}/Cu^{2+}-binding properties of clioquinol (see below) could explain the drug's effectiveness in inhibiting Aβ accumulation *in vivo*: CQ binding to Zn^{2+} facilitates the disaggregation and clearance of Aβ [40-42], and CQ binding to Cu^{2+} inhibits neurotoxic H_2O_2 production [43].

There could be a parallel here with the protective effects of Zn^{2+} and the recent suggestion that altered Cu^{2+} coordination converts Zn-deficient SOD (which normally binds copper and zinc simultaneously) from an antioxidant to a neurotoxic pro-oxidant [44]. Intriguingly, we have found that the coordination of Cu^{2+} by Aβ resembles the SOD1 active site, and that Cu binding generates an allosterically-ordered membrane-penetrating Aβ oligomer linked by SOD-like bridging histidine residues (see below) [45].

CQ treatment did not cause a systemic decrease in metal levels, probably because it is a relatively weak chelator (Ka is nanomolar for Zn^{2+} and Cu^{2+}), the metals being redistributed rather than excreted. It seems, therefore, that the benefits of the drug arise from its ability to bind selectively to the Aβ-metal complex, not from metal depletion of brain tissue. It is surprising that a chelator with such relatively low affinity for metals is so potent at inhibiting metal interactions with Aβ [46]. The hydrophobicity of CQ and possibly other stereochemical properties may facilitate the drug's access to the metal binding site on Aβ. If this were the case, then it is an important consideration in designing drugs based on CQ. We need to bear in mind, however, that Aβ has other, structurally undefined low affinity metal binding sites because it can bind up to 3.5 moles of Cu^{2+} and Zn^{2+} [38] per peptide subunit. These low-affinity metal binding sites on AβP may also be important for the pathophysiology of AD, and Cu^{2+} bound to these sites may be redox active and generate H_2O_2 [31, 45]. The affinity of CQ for Cu^{2+} is sufficient (nM) to dissociate Zn^{2+} and low affinity bound Cu^{2+} from AβP, and we have observed CQ inducing Cu^{2+} dissociation from Aβ by NMR spectroscopy [24]. Attempts to demonstrate this by EPR spectroscopy (Cu^{2+}:CQ would have a very different spectrum from Cu^{2+}:Aβ) have been frustrated by the low aqueous solubility of CQ. However, we have observed dissociation of Cu^{2+} from Aβ1−28 with the strong chelator EDTA, which also gives a very different EPR spectrum enabling the quantitation of Cu^{2+} bound to each entity (Curtain, unpublished). NMR failed to detect direct interaction of CQ with Aβ [24]. It is possible that CQ may act by reacting selectively with relatively low-affinity metals bound to Aβ, as well as by binding to the high affinity binding site perhaps by a ternary complex.

COPPER BINDING SITES AS METAL-BINDING DRUG TARGETS

Structural Considerations

Early solution NMR studies of human Aβ1−40 showed a random coil structure in aqueous solution (pH 4) at micromolar concentration [47]. Solution structures of Aβ1-40 in membrane-mimetic SDS-d_{25} micelles obtained by Coles *et al*. [48] showed two α-helical segments in the span from 17 to 40. The 'break' between the two helices was suggested by D_2O exchange experiments, where protons on residues 25-27 were shown to exchange rapidly, and from quantitative structural and dihedral angle restraint calculation prediction, a kink was seen at residues 26-27 acting as a 'hinge' for the two helices. Gröbner *et al*. [49] have outlined a method for structure determination of Aβ in membrane lipid systems. First, they used CD and ^{31}P Magic Angle Spinning (MAS) NMR to characterise the peptide in a DMPC/DMPG vesicle system. Their most notable finding at this stage was that they could get Aβ40 to give an α-helical structure if the peptide were dialysed from trifluoroethanol solution into the vesicles. Secondly, they used rotational resonance (RR) ^{13}C cross-polarisation MAS NMR recoupling techniques to show that the membrane-penetrant part of the peptide was α-helical, before major aggregation had occurred. These authors concluded, however, that future MAS studies would have to be made on multiple/uniformly labelled peptides. Thus, the determination of the structure of Aβ by NMR in a membrane environment is still a work in progress. The randomness of the peptide in aqueous solution, however, makes it difficult to determine the nature of the low affinity metal binding sites. Nevertheless, using a combination of NMR and EPR spectroscopy, which relied on the paramagnetic properties of Cu^{2+}, we have been able to determine the structure of the high affinity site and draw some conclusions about the interaction of the peptide with lipids and its modification by Cu^{2+}, Zn^{2+} and pH [45, 50]. It was found that in aqueous solution and lipid environments, coordination of metal ions to Aβ is the same, with His6, His13 and His14 all involved. The EPR spectrum of Cu^{2+} bound to the peptides has the unsplit intense g resonance characteristic of an axially symmetric square planar 3N1O or 4N coordination, $g_{\|}$ = 3.28 and g = 2.03 (Fig. **2A**). Electron spin echo modulation spectra (ESEEM) (Fig. **2B**) confirms that the coordination is 3N1O [51]. Since ESEEM spectroscopy is sensitive to coordination to the remote N of the His residues [51] this result suggests that these are involved in Cu^{2+} complexation. A notable finding was that increasing the Cu^{2+} above ~ 0.3M/ M peptide caused marked line broadening in the Cu^{2+} EPR spectra, suggesting the presence of Heisenberg electron exchange effects (Fig. **2C**). These effects could be explained if at Cu^{2+} /peptide molar ratios > 0.3, Aβ coordinated a second Cu^{2+} atom cooperatively. The effect was abolished if the histidine residues were methylated at Nε2, suggesting that bridging histidine residues, as found in the active site of SOD, were

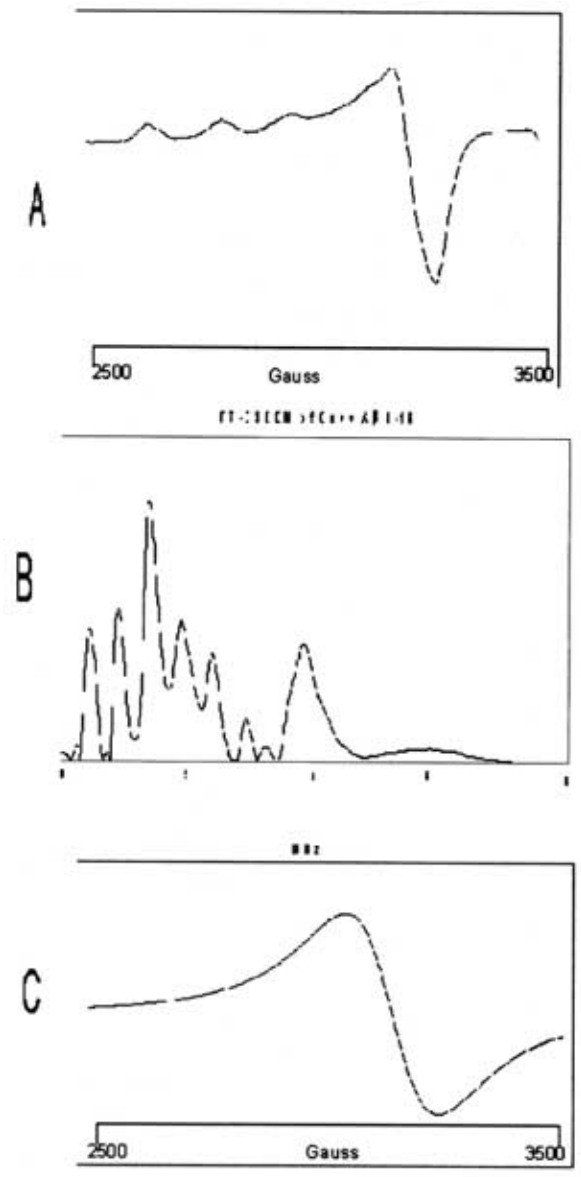

Fig. (2). A. EPR spectrum of 0.3 M Cu^{2+} complexed with 1.0 M Aβ1-28 in 20 mM pH 6.9 phosphate buffer. Width of spectrum 1000 Gauss, midfield 3000 Gauss, microwave frequency 9.57 GHz, temperature 77 K (after Curtain *et al.* [45]). Spectrum **2B**. Fourier transform of three pulse ESEEM spectrum of sample **A**. The height of the double quantum peak at 4.5 KHz is diagnostic of a 3N1O coordination [51]. Temperature 4 K, magnetic field 3050 Gauss, frequency, 9.57 GHz, pulse width 20 ns, τ = 180 ns, repetition rate 100 Hz. Spectrum **C**. is of an equimolar mixture of Cu^{2+} and Aβ1-28. Conditions as for Fig. **2A**.

being formed. Adding Cu^{2+} or Zn^{2+} to Aβ1-42 in the presence of negatively-charged large unilamellar vesicles altered the peptide conformation from β-sheet to α-helix, accompanied by oligomerisation and vesicle membrane penetration. It was concluded that metal binding to Aβ1−42 generates an allosterically ordered membrane-penetrating oligomer linked by SOD-like bridging histidine residues. The NMR evidence indicated that initial metal binding caused no significant structural change in the peptide, suggesting that metal-induced aggregation does not occur as a consequence of a metal-induced conformational change. Possibly, the metal could bridge between histidine residues of different peptides, as proposed by Miura *et al*. [52], but in this scheme the distance between copper ions would be > 8 Å, inconsistent with the 6 Å needed to account for the exchange interactions observed in the EPR spectra. It is also possible that histidine ligands could bridge between metal centres on different peptides. A consequence of binding of a metal ion to the Nδ1 of a histidine residue is reduction in the pK_a of Nε2 NH, making this nitrogen also available for metal binding [53], giving a histidine residue that can bridge metal ions; a good example being His63 at the active site of superoxide dismutase [54]. Similar bridging histidine residues have been proposed within the octarepeat region of the prion protein when it binds Cu^{2+} [55]; which has recently been shown to possess significant SOD activity in the presence of Cu^{2+} [56]. Miura *et al*. [52 showed complexes containing bridging histidine residues between Zn^{2+} and Aβ, but not between Cu^{2+} and Aβ. Figure (**3A**) shows how the histidine could act as a bridge between metal centres; the distance between copper ions in this model is 6 Å and exchange line broadening such as that shown in Fig. (**2C**) would be seen in the EPR spectra of such a complex. The bridging histidine is probably responsible for the reversible metal induced aggregation that is observed when Aβ is metallated with Cu^{2+} and Zn^{2+}. Dong *et al*. [23], using the spectra structure correlations for Aβ:transition metal binding, used Raman microscopy to study the metal binding sites in amyloid plaque cores. They observed that Zn^{2+} was coordinated to the histidine N_τ and the Cu^{2+} to the N_π . The bridging histidine residues also explain the multiple metal binding sites observed for each peptide and the high degree of cooperativity evident for subsequent metal binding [50]. With three histidines bound to the metal centre a large scope exists for metal-mediated cross-linking of the peptides leading to aggregation, which will be reversible when the metal is removed by chelation.

This type of metal binding with bridging histidine residues would result in complexes very similar to the active site of SOD. The occurrence of structured complexes, not merely random aggregates of peptide, was also suggested by the occurrence of exchange broadening in the Cu^{2+} EPR spectra of Aβ1−28 [45] and its modulation in Aβ1-40 and 1-42 by Zn^{2+} [50], which has also been observed in model SOD imidazolate-bridged dinuclear complexes [57]. In the case of Aβ1-28, there is no C-terminal hydrophobic region to be involved in such an association. Aside from the methylation experiments, the importance of the three histidines is shown by the fact that the rat sequence of Aβ does not reduce Cu^{2+} and Fe^{3+}, is not readily precipitated by Zn^{2+} or Cu^{2+} [33, 34, 58] nor produces ROS as avidly as the human sequence [3] One of the differences is that His13 in the human sequence is changed to an arginine in the rat, and Liu *et al*. [59] have shown that this residue is important for metal induced aggregation of Aβ peptides. The rat Aβ forms a metal complex via two histidine residues and two oxygen

ligands rather than three histidine residues and one oxygen ligand, compared with human Aβ where the side-chain of His13 of human Aβ is ligated to the metal ion. This was borne out by the EPR spectrum, which was typical of a distorted square planar 2N2O Cu^{2+} coordination.

Aβ in Cell Membranes

Being derived from the transmembrane region of APP, the Aβ peptides must inevitably be membrane associated in the neuropil and this must be taken into account in designing drugs directed at their structure. Indeed, ≈99% of total Aß in the normal human brain is incorporated into the membrane fraction [22, 60]. Although channels or pores have been observed with the Aβ [61], their significance to the pathophysiology of the disease is still unclear. The important issue is that the supramolecular structure of membrane-associated Aβ is unknown, although there are theoretical models for the structure of ion channels formed by membrane-bound Aβ40 [62]. Recently, Bhatia *et al.* and Lin *et al.* [63, 64] examined, using atomic force microscopy, Aβ1−42 reconstituted in a planar lipid bilayer and found 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. Using EPR and CD spectroscopy we [50] found that the presence of Cu^{2+} or Zn^{2+}, pH, cholesterol and the length of the peptide chain influenced the interaction of these peptides with lipid bilayers. In the presence of Zn^{2+}, Aβ40 and Aβ42 both inserted into the bilayer over the pH range 5-5.5, as did Aβ42 in the presence of Cu^{2+}. However, Aβ1−40 only penetrated the lipid bilayer in the presence of Cu^{2+} at pH 5.5; at higher pH there was a change in the Cu^{2+} 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 M 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, in accord with the SS-NMR findings of Gröbner *et al*. [49]. 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. Significantly, regardless of the location and conformation of Aβ, the copper binding site is not located in the lipid bilayer as evidenced by the lack of interaction between Cu^{2+} and paramagnetic probes embedded in the bilayer [42]. Nevertheless, since any Aβ metal site binding agent must be hydrophobic to cross the blood brain barrier, a large portion of it will be inevitably membrane associated. In these circumstances the stereochemical relationship between the hydrophobic region and the binding region could be quite critical.

Another potential copper binding target for AD therapy is the high affinity copper binding domain (CuBD) of APP. The APP molecule of AD has a CuBD located in the N-terminal cysteine-rich region which can strongly bind Cu(II) ($Kd_{APP} \approx 10$ nM) and reduce it to Cu(I) *in vitro* [65, 66]. *In vivo* studies show that APP expression modulates Cu homeostasis since APP knockout mice have increased Cu

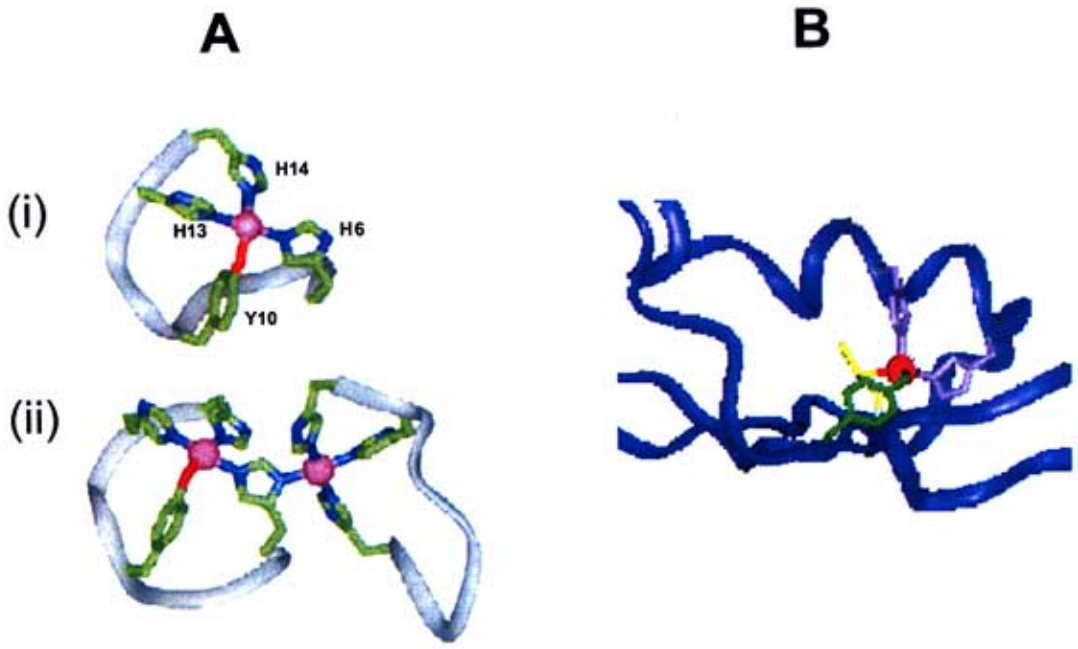

Fig. (3). Models of the coordination site(s) of Cu^{2+} bound to Aβ. **A**. The initial coordination site of Cu^{2+} in solution, (His6, 13 and 14 and Tyr10) as determined by NMR and EPR spectroscopy [45]. **B**. In this model explaining the aggregation, cooperative binding and redox properties of metal bound Aβ, the imidazole ring of His6 bridges the copper atoms forming a dimeric species. His6 is used as an example but other histidine residues could form similar bridges and therefore lead to aggregation. The coordination sphere about the metal ions is similar to that observed in the active site of SOD. **C**. The metal binding site of CuBD (Barnham *et al*. [72]). Orthogonal views of the putative metal binding site with Cu^{1+} coordinated in a tetrahedral configuration to H147, H151, Y168 and M170.

levels in the brain and liver [67]. The CuBD can modulate Cu(I) neurotoxicity [66] and depending on the ortholog can either promote or inhibit Cu-neurotoxicity [68] . The interaction between the APP-Cu(I) species with H_2O_2 results in Cu(I) oxidation to Cu(II) and APP fragmentation [69]. Most significantly, increasing the Cu concentration in a cell-line transfected with human APP cDNA modulates APP processing, resulting in greatly reduced Aβ production and increased levels of the cell-bound and secreted forms of APP [70]. Mutagenesis of histidine residues within CuBD inhibits the effects of Cu on APP expression and proteolysis [71]. Therefore, the interaction between APP and Cu has important consequences for Aβ production and AD pathogenesis. Agonists of copper interaction with APP would, therefore, have therapeutic potential. Accordingly, Barnham *et al.* [72] have determined the solution structure of CuBD, showing that it contains a novel Cu binding site consisting of ligands H147, H151, Y168 and M170 arranged in a tetrahedral fashion favoring Cu(I) coordination (Fig. **3B**). The tetrahedral coordination is supported by a Cu^{2+} EPR spectrum (Fig. **4**) characteristic of a tetrahedrally distorted planar spectrum with a 2N2S coordination. The surface location of this Cu-binding site and the role of the APP in Cu homeostasis could make the site an attractive target for drug design**.**

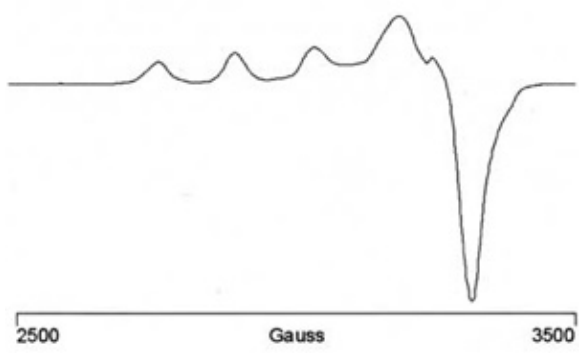

Fig. (4). EPR spectrum of 0.3 mM Cu^{2+} bound to 0.5 mM of CuBD in 20 mM pH 6.9 phosphate buffer. Same instrument settings as in Fig. **2A**.

CONCLUSION

The reaction of CQ with metallated Aβ in the brain has a number of curious aspects that are not yet fully understood. Any brain-targeting agent has to get across the cellular divide that constitutes the blood brain barrier [73]. The capillaries of the vertebrate brain and spinal cord are lined with a layer of special endothelial cells that lack the fenestrations found in other capillaries. Exchange, therefore, must take place transcellularly rather than non-specifically. This means that therapeutic agents cannot perturb these special capillary cells. Since copper and zinc play an important part in the economy of all cells special care must be taken to design agents targeting metal binding sites in the CNS that are very highly specific for these sites. Given these constraints, medicinal metallochemistry is developing as an important therapeutic option in the treatment of AD.

ABBREVIATIONS

Aβ	=	Amyloid β peptides, peptides of specific length referred to as $A\beta n_1\text{-}n_2$
APP	=	Amyloid precursor protein
CQ	=	Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline)
CuBD	=	Copper binding domain of APP
CD	=	Circular dichroism
DFO	=	Desferrioximine
DMPC	=	Dimyristoyl phosphatidylcholine
DMPG	=	Dimyristoyl phosphatidylglycerol
ESEEM	=	Electron spin echo modulation spectroscopy
ROS	=	Reactive oxygen species
SMON	=	Subacute myeloneuropathy
TETA	=	Tetraethyltriamine acetic acid

ACKNOWLEDGEMENTS

Supported with funds from the NHMRC, NIA (5RO1AG12686), and the Alzheimer's Association.

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