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Rapid Report

Detergent-like Interaction of Congo Red with the Amyloid β Peptide[†]

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ABSTRACT: Accumulating evidence links prefibrillar oligomeric species of the amyloid β peptide (Aβ) to cellular toxicity in Alzheimer's disease, potentially via disruption of biological membranes. Congo red (CR) affects protein aggregation. It is known to self-associate into micelle-like assemblies but still reduces the toxicity of Aβ aggregates in cell cultures and model organisms. We show here that CR interacts with Aβ(1−40) in a manner similar to that of anionic detergents. Although CR promotes β sheet formation and peptide aggregation, it may also solubilize toxic protein species, making them less harmful to critical cellular components and thereby reducing amyloid toxicity.

The link between the self-assembly of the amyloid β peptide (Aβ) and the progress of Alzheimer's disease is well-established, although the details of the molecular mechanisms and the pathological consequences are still debated. Accumulating evidence suggests that prefibrillar oligomeric Aβ species are likely to be responsible for the cellular toxicity, possibly via interaction with biological membranes (*1*−*3*). Several small molecules have been reported to modulate the oligomerization and/or fibrillization of several amyloidogenic peptides and proteins, including Aβ (*4*, *5*). For many of these compounds, the mechanisms of action are only vaguely understood. Among the most frequently studied of such molecules is Congo red [CR (Figure 1a)] (*6*). However, the details of its binding mechanism and influence on protein aggregation are still not well understood. At concentrations above ∼5 μM, CR self-associates into supramolecular complexes (*6*−*8*), and recent observations link the ability of CR to form such micelle-like structures to its effect on amyloid formation (*9*). Indeed, this property has been suggested to be generally important among at least some small molecule inhibitors of amyloid polymerization (*10*). On a biological level, CR and other potentially self-associating molecules have been shown to reduce the induced toxicity of aggregates of Aβ as well as of other misfolded peptides in cell cultures and model organisms (*6*, *11*, *12*). We here report results showing that CR interacts with Aβ in a manner very similar to that previously reported for anionic detergents (*13*−*15*). As a consequence of the interaction, β sheet structure is induced in Aβ and aggregation of the peptide is in fact promoted. On the basis of these results, we hypothesize that the mechanisms of action for self-associating small molecules (including CR) that reduce amyloid toxicity are not necessarily related to overall effects on protein aggregation. Instead, their detergent-like properties might modify the pathways of aggregation and/or solubilize toxic protein species and render them less prone to harmful interactions with critical cellular components.

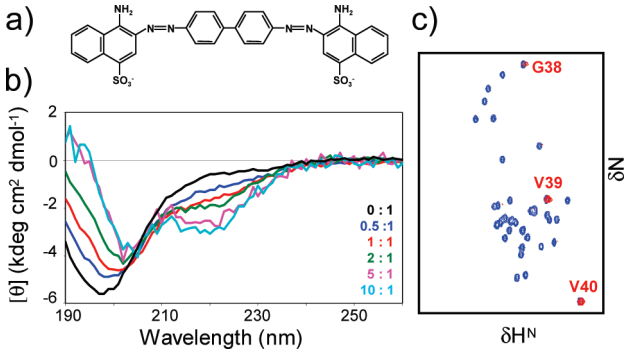

FIGURE 1: (a) Chemical structure of CR. (b) CD spectrum of 40 μM Aβ(1−40) in the presence of increasing concentrations of CR (0−400 μM) indicating formation of β structure. (c) $^{1}H-^{15}N$ HSQC NMR spectrum of 50 μM Aβ(1−40) in the absence (blue) and presence (red) of a 1 molar equiv of CR. Data were recorded at 3 °C in 10 mM phosphate buffer (pH 7.4).

Circular dichroism (CD) spectroscopy reveals significant alterations of the conformational landscape of Aβ(1−40) upon addition of CR (Figure 1b). The spectral features shift from those characteristic of random coil structure to those indicative of β structure. The isodichroic point observed in the titration indicates a structural transition between two main populations. A very similar effect has previously been described for the addition of low (1−2 mM) concentrations of SDS or LiDS to Aβ (*13*−*15*). The helical state observed for Aβ in the presence of high concentrations of SDS or LiDS cannot, however, be induced by CR in the concentration range investigated here.

Heteronuclear $^{1}H-^{15}N$ nuclear magnetic resonance (NMR) correlation spectroscopy was used to identify the regions of Aβ(1−40) interacting with CR. Addition of equimolar amounts of CR to 50 μM Aβ(1−40) results in substantial line broadening for the whole peptide sequence (Figure 1c). The residues at the extreme C-terminus are less affected than those of the rest of the sequence, and even at a 10-fold molar excess of CR, the two most C-terminal residues are visible (data not shown). The observed chemical shift changes indicate that the line broadening originates, at least partially, from chemical exchange processes on an intermediate time scale. The line broadening pattern is indeed very similar to that reported for Aβ with low concentrations of LiDS (*15*).

The nature of the Aβ(1−40)−CR complex was further investigated using pulsed field gradient diffusion (PFG) and saturation transfer difference (STD) NMR. The CR-bound state of Aβ cannot be directly observed by NMR, but as a result of the intermediate exchange rate, its properties influence the measured NMR observables. The diffusion rate of Aβ(1−40), as observed by PFG NMR, does not change significantly upon CR addition,

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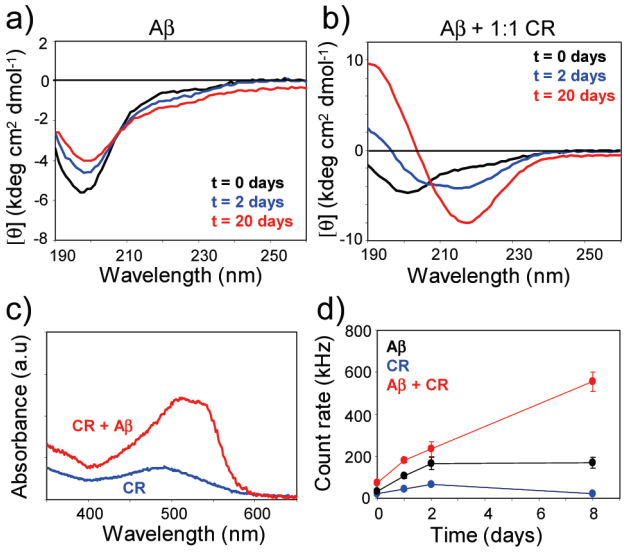

FIGURE 2: Time dependence of the structural properties of Aβ(1−40) incubated at 3−8 °C in 10 mM phosphate buffer (pH 7.4). (a and b) CD spectra of 40 μM Aβ in the absence (a) and presence (b) of a 1:1 molar ratio of CR. (c) The absorption spectrum of CR (same sample as in panel b) after incubation for 20 days is perturbed in a manner similar to that resulting from binding of the dye to amyloid fibrils. (d) DLS count rates indicate a more rapid formation of aggregated species in the sample with 40 μM Aβ(1−40) and 40 μM CR in 10 mM sodium phosphate buffer (pH 7.4) compared to controls with Aβ and CR alone.

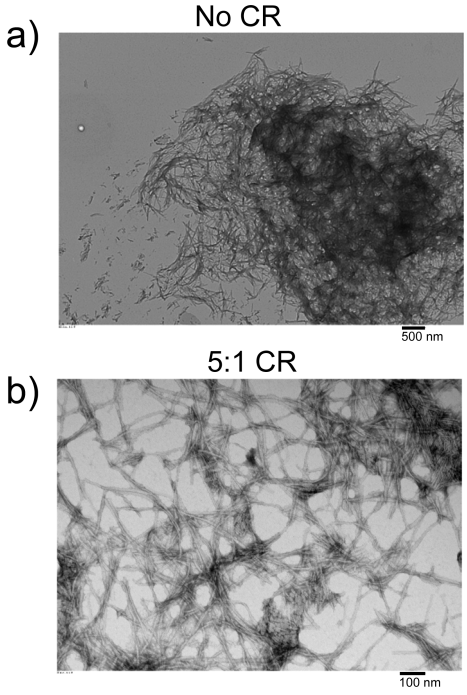

FIGURE 3: Transmission electron microscopy images of the species present at the end of the Aβ(1−40) aggregation assays (t = 24 h) in the absence (a) or presence (b) of a 5-fold molar excess of Congo red. The scale bars represent 500 nm (a) and 100 nm (b). The images were obtained at 4900× (a) and 14000× (b) magnification.

suggesting that the complex is not substantially larger than the free peptide (Figure S1a,b of the Supporting Information). STD NMR has previously been used to study Aβ self-association (*16*). We observe only minor changes in the STD NMR spectrum of Aβ(1−40) when adding increasing amounts of CR (Figure S1c,d of the Supporting Information), showing that the spin diffusion properties of the Aβ−CR complex are similar to those of the monomeric peptide, consistent with the results of the PFG NMR. Taken together, the NMR data suggest a binding mechanism in which one or a few CR molecules bind to Aβ and induce a slightly more compact (folded) conformation in the peptide. We cannot exclude the presence of a small fraction of larger peptide aggregates that cannot be detected by NMR because of slow exchange. However, the light scattering results (Figure 2d, vide infra) show that such aggregates do not represent a significant population.

Low concentrations of SDS have, like negatively charged lipid interfaces, been reported to promote Aβ self-assembly (*13*, *14*, *17*, *18*). Notably, micellar SDS rather inhibits the conversion of Aβ into β structure and peptide aggregation. Under the conditions of this study (~50 μM Aβ, ~5 °C, and no agitation), Aβ(1−40) by itself aggregates relatively slowly and we observed only minor changes in the CD spectrum over a period of 20 days (Figure 2a). In the presence of CR, a more rapid transition into β sheet structure is seen (Figure 2b). This observation is corroborated by a characteristic shift in the absorbance spectrum of CR bound to amyloid-like β structure (Figure 2c) (*6*). Enhanced formation of aggregates in the presence of CR was also observed using dynamic light scattering (DLS) (Figure 2d). Aggregation of Aβ(1−40) and Aβ(1−42) was further studied at a higher temperature (29 °C) and with continuous agitation, and again CR accelerated the process (Figure S2 of the Supporting Information). Notably, while CD clearly showed an increased level of formation of β structure in the presence of CR, traditional thioflavin T fluorescence did not detect amyloid formation for these samples (Figure S2); this could result from interference between the two amyloid binding dyes (*9*). Transmission electron microscopy images of the aggregates do, however, confirm the formation of fibrillar peptide aggregates in the presence of CR (Figure 3 and Figure S3 of the Supporting Information). Hence, CR accelerates the process of β structure formation and peptide aggregation in a manner similar to that observed for anionic detergents.

Taken together, our data reveal clear similarities between the interactions of Aβ with CR and low concentrations of SDS or LiDS. In both cases, NMR signal intensity is lost for the whole peptide sequence except for the very C-terminal residues (Figure 1c). We also find that CR initiates or promotes the formation of β structure (Figure 2b) and higher-order Aβ species which give rise to increased light scattering (Figure 2d), and that CR remains bound to this form of Aβ (Figure 2c).

It is intriguing that CR, with two negative charges, and the anionic detergents and lipids interact with the negatively charged Aβ peptide. A clue to this puzzle can be found in the structure of a β hairpin that can be stabilized in the monomeric Aβ peptide (*19*). In this structure, two lysine residues (K16 and K28) are flanking the two strands of the intramolecular β hairpin and the distance between these lysines is in good agreement with the overall dimension and charge separation in CR (Figure S4 of the Supporting Information) (*6*). Electrostatic interactions between the two charge pairs could therefore perhaps promote the folding of Aβ into a hairpin with intramolecular antiparallel β structure, and this species might seed the formation of larger aggregates. The headgroups of anionic detergents and lipids may have similar roles in promoting intramolecular folding of Aβ. The proposed structural model would indeed explain the strong intersulfonate distance dependence previously reported for the binding of Aβ to different sulfonated dyes (*20*).

Our observations differ from previous reports of CR as an inhibitor of protein aggregation (e.g., refs *4*, *5*, *10*, *11*, and *21*). One explanation for this discrepancy could be the selection of the experimental approach. For example, CR has recently been shown to interfere with thioflavin T fluorescence as an indicator of amyloid formation (*9*), and we here observe a similar effect. The existence of independent aggregation pathways might also explain why certain types of aggregates are not observed, although self-association is not completely inhibited (*4*). Furthermore, the detergent-like properties of CR make the compound concentration critical for the outcome of the experiments. The effect on protein aggregation might be different below and above the critical concentration of self-association. This is indeed an important issue since self-assembly has been reported for many small molecules (*10*).

Our findings raise the question of whether the reported ability of CR to reduce the cellular or in vivo toxicity induced by Aβ and other amyloidogenic peptides (*6*, *11*, *12*) could in fact be explained by mechanisms other than a reduced overall level of peptide aggregation. CR appears to promote the formation of specific types of aggregates that are less toxic than those formed in its absence. The exact structural features that could reduce the toxicity are not clear; however, if the compound acts like a detergent, it may modify the surface properties of the protein species. Such a mechanism could indeed explain the weakened interaction of the Aβ oligomer-specific A11 antibody with Aβ aggregates formed in the presence of CR (*4*). The reduction of amyloid cytotoxicity associated with CR might thus be related to the solubilization of harmful oligomeric species and inhibition of their interaction with cellular components, such as membranes. A similar mechanism has been suggested for small molecules that modulate the electrostatic properties of membranes (*22*). Indeed, CR has been reported to reduce Aβ-induced toxicity by addition to preformed amyloid aggregates (*11*) and also to inhibit Aβ-induced Ca^{2+} leakage (*23*) as well as pore formation by several amyloidogenic proteins (*24*). Furthermore, glycosaminoglycans located at the cell surface have been suggested as potential nucleation sites for oligomerization of Aβ and other peptides and proteins (*3*, *6*). The presence of CR, which possesses some similarities to the glycosaminoglycans, could then provide alternative and competitive nucleation sites and thereby keep harmful oliomeric species away from membranes.

In conclusion, our observations strongly suggest that detergent-like properties of small molecule effectors of protein or peptide aggregation, like CR, should be taken into account when investigating their mechanisms of action in efforts to design small molecule therapeutics for amyloid disorders such as Alzheimer's disease.

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SUPPORTING INFORMATION AVAILABLE

Experimental protocols, PFG and STD NMR data, Aβ(1−40) and Aβ(1−42) aggregation assays, TEM images of Aβ(1−40) and Aβ(1−42) aggregates, and a model for the CR−Aβ interaction. This material is available free of charge via the Internet at http://pubs.acs.org.

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