Structural features of the Cu(II) complex with the rat Aβ(1–28) fragment†

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The interaction between Cu(II) and the rat amyloid β (1–28) fragment in micellar solutions at pH 7.5 was investigated by CD and NMR spectroscopy; the proton–copper distances were used in restrained molecular dynamics simulations to obtain a structural model of the Cu(II) complex.

Amyloid β (Aβ) is a peptide of 39–43 amino acids originated by proteolysis of the amyloid precursor protein (APP).[1–3] Aβ(1–42) is remarkably prone to self-association[4] with production of large, insoluble extracellular deposits of β sheet amyloid fibrils found in neuritic plaques in the brain of individuals affected by Alzheimer's disease (AD).[5,6] The interaction of metals with Aβ plays a pivotal role in the pathogenesis of Alzheimer's disease (AD).[7–9] Transition metals, such as Cu(II), Zn(II), and Fe(III), are found in Aβ plaques, and they have been shown to affect Aβ aggregation *in vitro*.[10] The Cu(II)–Aβ complex has been studied by different spectroscopic techniques such as EPR, Raman, and NMR revealing that the metal binding site is located at the N-terminus, within the first 16 residues. All investigations have concluded that Aβ coordinates the metal through the three His residues His-6, His-13, and His-14[11–21] but different results have been reported as for the fourth ligand; Tyr-10 phenolate,[11,12,14,15] Asp-1 N-terminal nitrogen,[16–18] or an as yet unidentified carboxylate side chain[19,20] have alternatively been suggested as additional binding donors. Cerebral Aβ deposition is encountered in many aged mammals[22] but not in aged rats;[23,24] the rat Aβ(1–40) contains three point substitutions, R5G, Y10F and H13R, that appear to alter the physicochemical properties of the peptide preventing its precipitation in the brain.[24,25] Here we present NMR and molecular dynamics results obtained on the rat Aβ(1–28) fragment (DAEFGHDSGFEVRHQKLVFFAEDVGSNK-NH_2, hereafter called rAβ) and its Cu(II) complex as a means to characterize the rat Aβ–Cu(II) interaction and to solve the 3D structure of the paramagnetic complex.

The ^{1}H 1D NMR spectra of an aqueous millimolar solution of rAβ at pH 7.5 revealed that some aggregation was occurring within a few hours from sample preparation, as observed from large broadening of all proton resonances, such that lengthy NMR experiments (10 hours or more) were not affordable. The peptide was therefore dissolved in H_2O–SDS (sodium dodecyl sulfate 0.1 M) solutions, a membrane mimicking environment reported to induce the formation of two α-helices in the C-terminal region of Aβ peptides (residues 15–24, and 29–35, respectively);[26–30] such conformational preference is thus expected to prevent β sheet formation and the consequent aggregation of the peptide. SDS micelles yielded soluble and stable samples for many days and induced α-helix structuring also in rAβ, as pointed out by the analysis of the NOESY and CD spectra (see ESI†). The SDS–rAβ interaction was also checked by diffusion-ordered NMR spectroscopy (DOSY) experiments.

Addition of increasing amounts of Cu(II), from 0.05 to 0.5 equivalents, at pH 7.5 caused selective proton line broadening mainly affecting signals of the histidines and of the N-terminal region (Asp-1 and Ala-2) as shown in Fig. 1. In particular, starting from the addition of 0.1 metal equivalents, all NMR signals belonging to residues located between the N-terminus and the two His were completely washed out from the spectrum, preventing the measurement of relaxation rates. Proton longitudinal relaxation rates were therefore measured for the free peptide (R_{1f}) and in the presence of only 0.05 equivalents of the metal ion (R_{1obs}), to obtain the paramagnetic relaxation enhancements (R_{1p}) from the following equation:[31,32]

$$R_{1p} = R_{1obs} - p_f R_{1f} = \frac{p_b}{R_{1b}^{-1} + \tau_M} \quad (1)$$

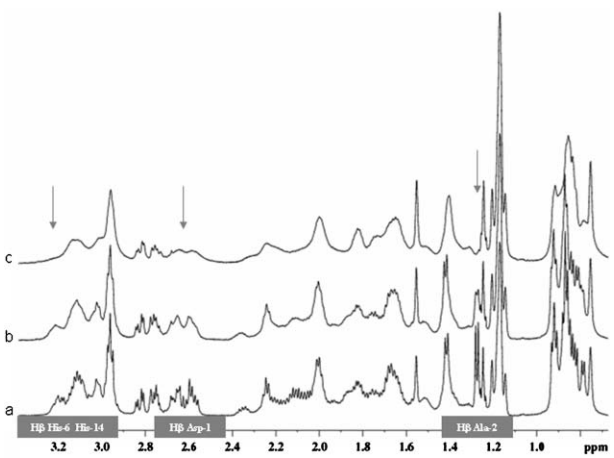

Fig. 1 Aliphatic region of 1D ^{1}H NMR spectra of rAβ 2 mM, SDS 0.1 M, pH 7.5, *T* 318 K. (a) Free peptide; (b) in presence of 0.05 Cu(II) eq; (c) in the presence of 0.5 Cu(II) eq. The arrows indicate the most affected protons.

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† Electronic supplementary information (ESI) available: Peptide synthesis, NMR and CD measurements, molecular dynamics calculations, temperature dependence of R_{1p} of His-6 and His-14, CD spectra of rAβ in the absence and in the presence of Cu(II), Cu(II)–H distances calculated with different τ_M values. See DOI: 10.1039/b713453c

where $\tau_M = k_{off}^{-1}$ is the mean residence time of peptide molecules in the metal coordination sphere, p_f and p_b are the fractions of free and bound peptide, and R_{1b} is the relaxation rate of ligand nuclei in the metal coordination sphere.

The effect of the paramagnetic ion was monitored within the 303–318 K temperature range and it was found, as expected by the τ_M increasing at lower temperatures,[32] that the higher the temperature the larger were the paramagnetic relaxation enhancements, especially those on His aromatic protons (ESI Fig. S1†). The R_{1p} of all the other protons were therefore calculated at T 318 K (Fig. 2). It is evident that Asp-1 and the two His are the most affected residues, which supports copper binding to His imidazoles and to Asp-1. In particular the largest effects monitored on Asp-1 Hα indicate the metal coordination to the N-terminal nitrogen but do not exclude the additional involvement of Asp-1 carboxylate, which has been reported for both human and rat Aβ(1–16) fragments either *via* a direct covalent bond[33] or *via* hydrogen bonding with an axial bound water molecule.[18]

The absence of relevant effects on Hα relaxation rates of residues preceding and following the two histidines suggests that copper binding does not result in deprotonation of amide nitrogens. The negligible R_{1p} values calculated in the C-terminal region (Fig. 2, from residue 16 to 28) exclude its proximity to the Cu(II) binding site suggesting that such region is not perturbed by the metal rAβ complexation. The NMR diffusion experiments were also performed in the presence of the metal (0.5 eq.) and an almost identical diffusion coefficient was found indicating the persistence of the peptide–micelle interaction after the Cu(II) binding. In fact the N-terminal region of the peptide, located outside the micelle, is free to interact with different metal ions such as Cu(II),[11] Zn(II)[11] and Mn(II).[30] The CD spectra performed on the amyloid fragment either in the presence or in the absence of the paramagnetic ion (ESI Fig. S2†) indicate the presence of an α-helix conformation also upon Cu(II) binding. All these findings, (i) the neglibible R_{1p} effects, (ii) the conservation of the diffusion coefficients and (iii) the evidence of some α-helix structure lead us to suppose that the C-terminal region of the rAβ still interacts with SDS and retains its α-helix structure in the Cu(II)–rAβ complex. Such interaction, moreover, excludes the existence of bis Cu(II) complexes also at the very low metal peptide ratio ($p_b = 0.05$) used for the calculation of the paramagnetic relaxation enhancements.

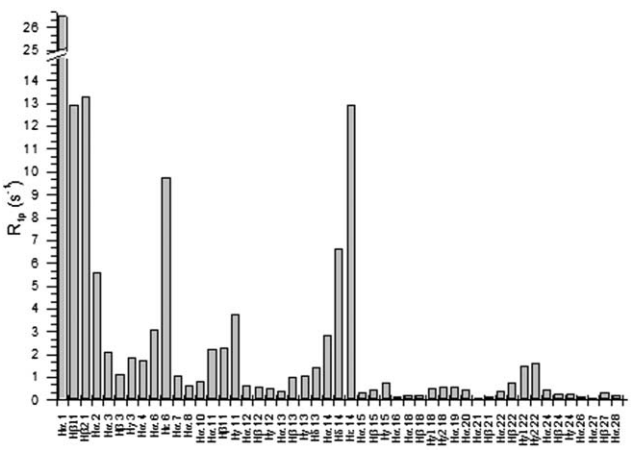

Fig. 2 ^{1}H paramagnetic relaxation enhancements of rAβ 2 mM, SDS 0.1 M, pH 7.5, T 318 K calculated in the presence of 0.05 Cu(II) eq.

Experimental R_{1p} values allow calculation of copper–proton distances by using the Solomon equation,[34] describing the dipolar nuclear spin–electron spin interaction. In order to make this possible, the values of τ_M and of τ_R, the rotational correlation time modulating the interaction, must be known. Estimates of τ_M were obtained by the combination of eqn (1) and the Solomon equation, considering $\tau_R = 4.3$ ns (calculated from the Stokes equation), and using the three metal–proton distances arising from copper coordination to Asp-1 amino nitrogen and to both His imidazoles. In particular, the Cu(II)–Hα distance, with copper binding to the N-terminus, can range from 0.25 to 0.40 nm,[35] while the Cu(II)–Hε distance is fixed to 0.31 nm, with Cu(II) binding either to Nδ or Nε.[36,37] These three distances, concerning Asp-1, His-6 and His-14, together with the corresponding R_{1p} values, yielded a τ_M of 1.7 ms, 5.0 ms and 3.8 ms at T 318 K, respectively.

The obtained values of τ_M are of the same order of magnitude and they give rise to comparable metal–proton distances, with differences between the maximum and minimum value of each proton within the range used for structure calculation for all but two protons (ESI Fig. S3†); the average of these three values was therefore used for calculating all metal–proton distances within the Cu(II)–rAβ complex. Moreover, the R_{1p} of His-6 and His-14 Hδ yields Cu(II)–proton distances of 0.53 and 0.49 nm respectively, which are consistent with copper binding to the Nδ imidazole nitrogen in both His residues.

All the calculated distances were then used as constraints for structure determination of the Cu(II)–rAβ complex, together with distances derived from NOESY spectra. The best obtained structure was optimized through an energy minimization followed by a molecular dynamics simulation in water–SDS (Fig. 3). The RMSD calculated on copper and its coordinating residues for all the reported structures is of 0.04 nm. The {$2N^{Im}$, NH_2} donor set

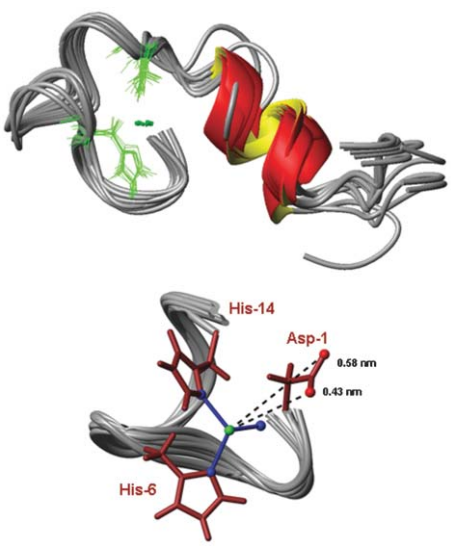

Fig. 3 Snapshots from the MD simulation of the Cu(II)–rAβ complex: (upper) the backbone is shown as a ribbon, the copper ion and the two coordinating histidines in green; (lower) the Cu(II) binding region: the Asp-1, His-6 and His-14 nitrogen donors (blue spheres) bound to Cu(II) (green sphere) and the distances Cu(II)–COO^- from Asp-1 are shown.

verified by the obtained structure matches the binding mode proposed for Cu(II) interacting with the human peptide,[18–20] with the exception of the involvement of His-13. It is therefore His-13 that is very likely to determine the different biophysical properties of the human and rodent peptides, such as the reduced tendency of rat Aβ to aggregate. In the case of Cu(II)–rAβ complex, a water molecule might substitute the His-13 imidazole binding to the paramagnetic ion. From the obtained structure, in fact, the direct involvement of Asp-1 side chain carboxylate can be excluded being 0.43–0.58 nm far away from the metal center. The Asp-1 carboxylate interaction *via* hydrogen bonding with a water molecule, as found for the human Aβ fragments,[18] can therefore be hypothesized also for the rat peptide. The similarity of human[18] and the rat Aβ copper coordination sphere seems to attribute to His-13 (missing in the rat sequence) a predominant role in Aβ accumulation which can be due to the strong copper binding ability of the His-13–His-14 pair which, close to the highly hydrophobic region encompassing residues 16–24, might induce relevant conformational changes.

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