Supplementary Material (ESI) for Chemical Communications

Structural features of the Cu^{2+} complex with the rat Aβ(1-28) fragment

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SUPPLEMENTARY INFORMATION

Peptide synthesis and purification – Synthesis of rat amyloid 1-28 fragment was performed on a solid-phase using Fmoc (Fmoc = 9-fluorenylmethoxycarbonyl) strategy with continous-flow methodology (9050 Plus Millipore Peptide Synthesizer) on a polystyrene/polyethylene glycol copolymer resin (TentaGel R RAM Resin, substitution 0.18 mmol/g) (1). Attachment of the first amino acid to the resin and next coupling steps were realized using diisopropylcarbodiimide (DIPCI) as a coupling reagent with 1-hydroxybenzotriazole (HOBt) as an additive. Removal of Fmoc protecting group during peptide synthesis was achieved by means of 20% piperidine solution in DMF / NMP (1:1, v/v) with addition of 1% Triton X-100 (1). The peptides was cleaved from the resin and deprotected by treatment with the mixture containing trifluoroacetic acid, phenol, triisopropylsilane and water (88:5:2:5, v/v), for 2h at room temperature (1). The resulting crude peptide was purified by reversed-phase high-performance liquid chromatography (RP-HPLC) using a C8 semi-preparative Kromasil column (25 × 250mm, 7μm). The purity of the peptide was confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and analytical RP-HPLC using a C4 Kromasil column (4.6 × 250mm, 5μm) and 30 min. linear gradient of 0-80% acetonitrile in 0.1% aqueous trifluoroacetic acid as a mobile phase. Analytical data were as follows: rAβ(1-28): Rt = 15.2 min, MS = 3164.6 $[M+H]^+$, Mcalc = 3163.4. The peptide was first dissolved in H_2O or D_2O, followed by the addition of SDS-d_{25} (Sigma Chemical Co.). The SDS-d_{25} concentration was kept high relative to the peptide concentration, so that it was well above 8 mM (the critical micelle concentration) (2) and also above the average aggregation number. The pH values were measured at room temperature and they were adjusted at desired values with either DCl or NaOD solutions. The desired concentration of copper ions was achieved by using a stock solution of copper(II) nitrate (Sigma Chemical Co.) either in water or deuterium oxide. TSP-d_4, 3-(trimethylsilyl)-[2,2,3,3-d_4] propansulphonate, sodium salt, was used as internal reference standard.

CD experiments: CD spectra were obtained with a Jasco J-815 spectropolarimeter and the temperature was controlled with PTC-423S temperature controller. A quartz cell with 1 cm optical path was used. The spectra range was 190-250 nm with a resolution of 0.2 nm and a bandwidth of 1

nm. A scan speed of 20 nm/min with 1 s response time was employed. The background spectrum was subtracted and the results were expressed as mean residue molar ellipticity [Θ].

NMR experiments: NMR experiments were carried out at 14.1 T or at 9.4 T at controlled temperature (± 0.1 K) on a Bruker Avance 600 MHz or Bruker AMX 400 MHz spectrometer equipped with a Silicon Graphics workstation. Suppression of residual water signal was achieved either by presaturation or by excitation sculpting, (3) using a selective square pulse on water 2 ms long. Proton resonance assignment was obtained by COSY, TOCSY, NOESY and ROESY experiments. Proton spin-lattice relaxation rates ($R_1 = 1/T_1$) were measured with the standard inversion recovery pulse sequence; relaxation rates were calculated with regression analysis of the initial recovery curves of longitudinal magnetization components, leading to errors in the range ± 3%. While the simple inversion recovery experiment is suitable for the well-isolated peaks, the IR-TOCSY sequence was used to calculate the relaxation rates of the overlapping ^{1}H NMR signals (4). This was obtained by introducing a ^{1}H 180° pulse followed by a variable delay in front of the TOCSY sequence. The T_1 values were determined by a three-parameter fit of peak intensities to the following equation:

$$I(\tau) = I_0[1 - (1 + B)\exp(-\tau / T_1)]$$

where B is variable parameter that considers non ideal magnetization and which value is smaller than one. The obtained results were compared with those obtained from normal IR sequence. The agreement was found in the errors limit of both experiments. The diffusion coefficients were measured at 298 and 318 K by a PFG longitudinal eddy-current delay (LED) pulse sequence with bipolar gradients incorporating spoil gradients during both longitudinal storage periods (5-8). The gradient strength was incremented (with an initial value of 0.86 G cm-1 and a step size of 2.65 G cm-1 for 2 ms), while the separations of the field gradients and the total echo time were kept constant. A series of 16 spectra, with a number of scans ranging from 16 to 32, was recorded in 2D mode for each measurement, with a recycle time of 10 s between scans. The diffusion values were calculated by regression analysis of the signal decay leading to errors not larger than ± 2-5%. The strength of the B_0 field gradient was calibrated by measuring the self-diffusion coefficient of the residual HDO signal in a 100% D2O sample at 298 K (9,10).

Structure determination and molecular dynamics simulations: For the free rAβ peptide, the intensities of NOESY cross-peaks, referenced to cross-peaks related to proton pairs at fixed distances, were converted into proton-proton distance constraints. For the Cu(II)-rAβ complex, all

R_{1p} values, obtained from NMR measurements, were converted into metal-proton distance constraints; the conservation, in the C-terminal region, of the helical secondary structure already observed in the free form, indicated by experimental data, was explicitly imposed during the structure calculation. These constraints were used to build a pseudopotential energy for a restrained simulated annealing (SA) calculation in torsional angle space. In particular, we performed the calculation with the program DYANA (5), with 300 random starting structures of the complex, and 10000 steps of SA. Since only one molecule can be given as input in the program, the peptide was linked to Cu(II) through a long chain of linkers, *i.e.*, residues made by atoms without van der Waals radius, which could freely rotate around their bonds, without causing steric repulsions, and thus enable one to sample a large number of relative positions of the ligand with respect to the metal ion before the minimization step.

On the best structure of the Cu(II)-rAβ complex obtained with this procedure, we performed an energy minimization followed by a molecular dynamics (MD) simulation, using the program GROMACS (6, 7) with the OPLS force field (8). First, the structure was energy minimized *in vacuo*, then it was solvated using a parallelepiped box of water with periodic boundary conditions, with a minimum distance between any atom of the peptide and the box edge of 1.5 nm, in the presence of a pre-equilibrated SDS micelle, and of a number of Na^+ counterions such as to balance the negative charge of the micelle. The peptide was positioned so as to place its C-terminal α-helical region near the external surface of the micelle, in agreement with previous studies showing that the "whole" Aβ(1–40/42) peptide interacts with SDS micelles at their surface, rather than within their hydrophobic core. The resulting system was again energy minimized and subsequently brought to the temperature of 298 K through six MD runs of 10 ps each, in which the temperature was progressively raised. Then an MD simulation of 400 ps at constant temperature (T=298 K) was performed. During the simulation peptide, micelle and solvent separately were weakly coupled to a temperature bath at the chosen temperature and to a pressure bath at 1 atm, with relaxation times τ_T = 0.1 ps and τ_P = 0.5 ps, respectively, using Berendsen's weak coupling algorithm (9); bond lengths were constrained to equilibrium values using the SHAKE procedure (10), with a geometric tolerance of 10^{-4}, and the time step was set to 2 fs. Nonbonded interactions were treated using a twin range method (11): within a short-range cut-off of 0.8 nm all interactions were determined at every time step, while longer range contributions within a cut-off of 1.4 nm were evaluated each time the pair list was generated. Distance restraints of 0.2 nm between the metal ion and its coordinating nitrogens were imposed.

In order to build the micelle, first we added the single SDS molecule topology to the chosen force field, then we arranged 50 SDS molecules in a roughly spherical shape; this initial micelle

was subsequently equilibrated through an energy minimization and a molecular dynamics simulation in water with an appropriate number of Na^+ ions, imposing distance constraints between each of the terminal carbons of the hydrophobic tails and a dummy atom placed at the center of the micelle.

References:

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Figure Captions

Figure S1. Temperature dependence of R_{1p} values calculated on His-6 and His-14 Hε of 2 mM rAβ(1-28) in D2O, 100 mM sds, pH 7.5.

Figure S2. CD spectra of 6 μM rAβ(1-28) in absence (lower trace) and in presence (upper trace) of 1.0 Cu^{2+} eq. in H_2O/SDS solutions, pH 7.5 at 318 K.

Figure S3. Proton-copper distances of selected protons of Cu(II)- rAβ(1-28) complex, calculated with different τ_M values (1.7- 3.8- 5.1 ms).

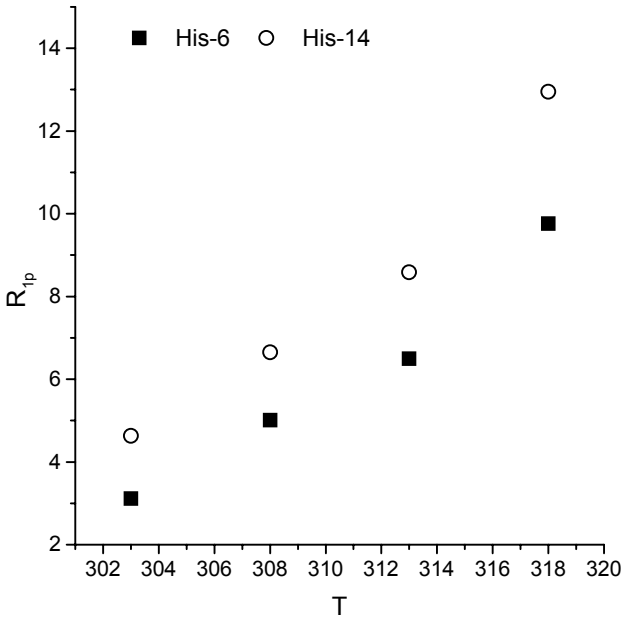
His-6
His-14
R_{1p}
T
2
4
6
8
10
12
14
302
304
306
308
310
312
314
316
318
320

Figure 1S

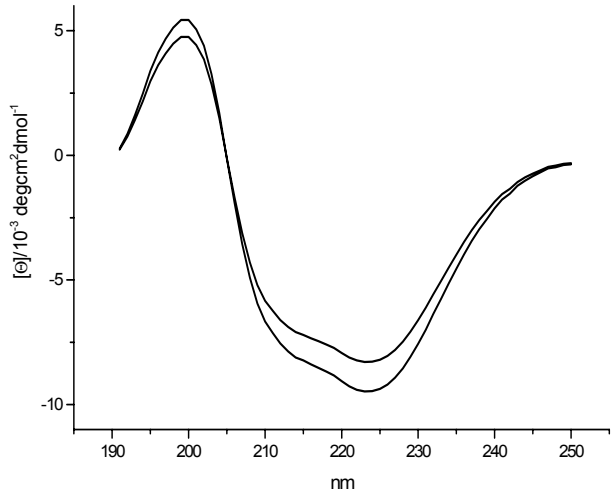

Figure S2

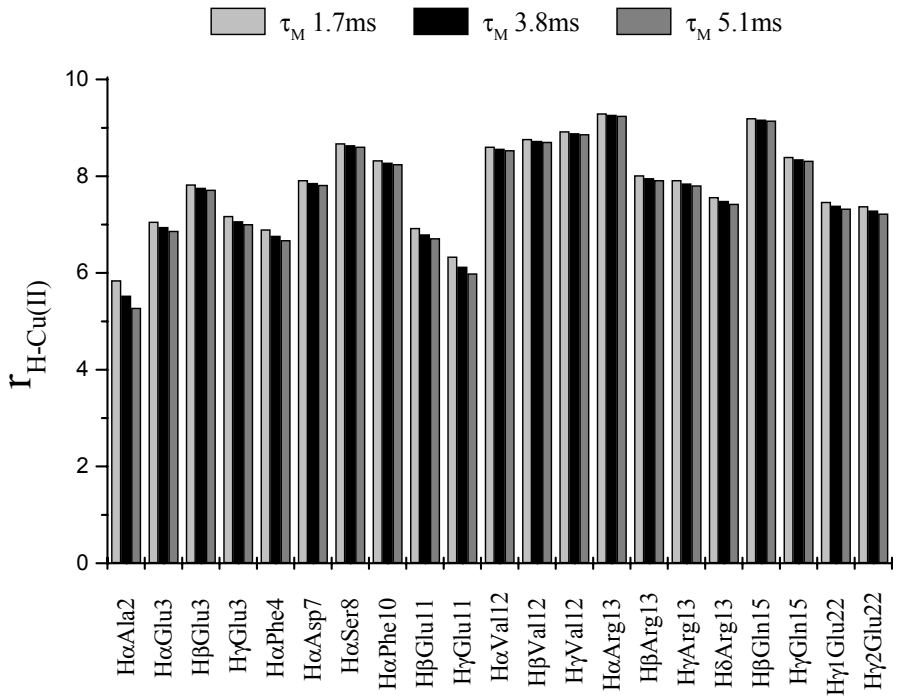
τM 1.7ms
τM 3.8ms
τM 5.1ms
r H-Cu(II)
0
2
4
6
8
10
HαAla2
HαGlu3
HβGlu3
HγGlu3
HαPhe4
HαAsp7
HαSer8
HαPhe10
HβGlu11
HγGlu11
HαVal12
HβVal12
HγVal12
HαArg13
HβArg13
HγArg13
HδArg13
HβGln15
HγGln15
Hγ1Glu22
Hγ2Glu22

Figure S3