Supporting Information

Bioinorganic Chemistry of synucleinopathies: Deciphering the binding features of Met motifs and His-50 in AS-Cu(I) interactions

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Protein and reagents. The protein AS was prepared as previously described [S1]. The M116A AS and M127A AS mutants were constructed using the Quick-Change site-directed mutagenesis kit (Stratagene) on AS sequence-containing plasmids. The introduced modifications were further verified by DNA sequencing. Purified proteins were dialyzed against Buffer A (20 mM MES, 100 mM NaCl, pH 6.5) supplemented with Chelex (Sigma). The peptide ^{45}KEGVVHGVATV^{55} (*aa*45-55 AS) was synthesized in solid-phase (Rink amide resin) using F-moc chemistry. Peptide was acetylated at the α-NH_2 terminal and amidated at the C-terminal carboxylate group. The peptide was purified, characterized and quantified as previously described [S2].

Generation of the Cu(I) Complexes. To obtain the Cu(I) complexes with AS/peptide variants (50-100 μM), the Cu(II) complexes were first prepared and then reduced with 6-12 mM of ascorbic acid, which was added from a 0.5 M stock solution. After pH adjustment, samples were treated with a flow of N_2 during 5 minutes to generate an N_2 atmosphere. NMR tubes sealed under N_2 atmosphere were used in all cases.

Reduction of Cu(II)-protein complexes with ascorbic acid, as shown in equation 1, was followed by the decrease of the characteristic d-d transition band in the UV-Vis spectrum, as previously described [S2, S3]. Spectra were recorded at 15°C on a Jasco V-550 spectrophotometer.

$$\text{Ascorbic Acid} + 2\ \text{AS-Cu(II)} \rightarrow \text{Dehydroascorbic Acid} + 2\ H^+ + 2\ \text{AS-Cu(I)} \quad (1)$$

NMR spectroscopy. NMR spectra were acquired on a Bruker Avance II 600 MHz spectrometer using a triple-resonance probe equipped with z-axis self-shielded gradient coils. 1D ^{1}H and 2D ^{1}H-^{15}N HSQC NMR experiments were recorded in Buffer A with 10% D_2O at 15ºC. ^{1}H-^{15}N HSQC cross-peaks and ^{1}H NMR resonances affected during metal titration experiments were identified by comparing their chemical shift

values with those of the same cross-peaks in the data set of samples lacking metal ion. Differences in the mean weighted chemical shift displacements (MWΔCS) for ^{1}H-^{15}N were calculated as $[(\Delta\delta^{1}H)^2 + (\Delta\delta^{15}N/10)^2]^{1/2}$ [S3].

The affinity features of AS-Cu(I) complexes were determined from ^{1}H-^{15}N HSQC experiments on 100 μM protein samples recorded at increasing concentrations of the metal ion. Changes in chemical shifts values of amide resonances were fit to a model incorporating complexes of Cu(I) into three classes of independent, non-interactive binding sites (with an apparent dissociation constant K_d), using the program DynaFit [S4]. The reported K_d values assume that Cu(I) disproportionation to Cu(II) and/or precipitation of Cu(OH) and Cu_2O did not occur or were extremely low under the experimental conditions of our studies. The absence of line broadening in the NMR spectra, indicative of the formation of paramagnetic Cu(II) ions or molecular precipitates, support this assumption.

One-bond N–H Residual Dipolar Couplings (RDCs) were measured on ^{15}N-AS samples aligned in 5% (w/v) n-octylpenta(ethylene glycol)/octanol (C8E5) [S5,S6]. Spectra were acquired by use of the 2D inphase-antiphase (IPAP)HSQC sequence under both isotropic and anisotropic conditions [S7].

Acquisition, processing, and visualization of the NMR spectra were performed using TOPSPIN 2.1 (Bruker) and CPP-NMR.

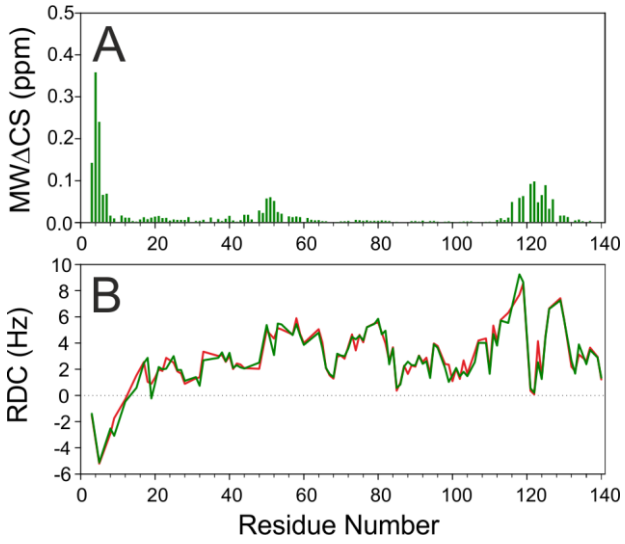

Figure S1. RDC profile and chemical shifts displacements of backbone amide groups of AS-Cu(I) complexes. (A) The panel shows the differences in the mean weighted chemical shifts displacements between free and Cu(I)-complexed AS at a molar ratio of 3:1. Mean weighted chemical shifts displacements (MWΔCS) for ^{1}H and ^{15}N of amide resonances were calculated as $[(\Delta\delta^{1}H)^{2} + (\Delta\delta^{15}N/10)^{2}]^{½}$. (B) RDCs were measured on ^{15}N-AS samples aligned in 5% (w/v) *n*-octylpenta(ethylene glycol)/octanol (C_8E_5). RDC measurements were measured in the absence (green) and presence (red) of 3 equivalents of Cu(I) ions. All experiments were recorded on 100 μM protein samples dissolved in buffer MES 20 mM, NaCl 100 mM, pH 6.5 at 15ºC.

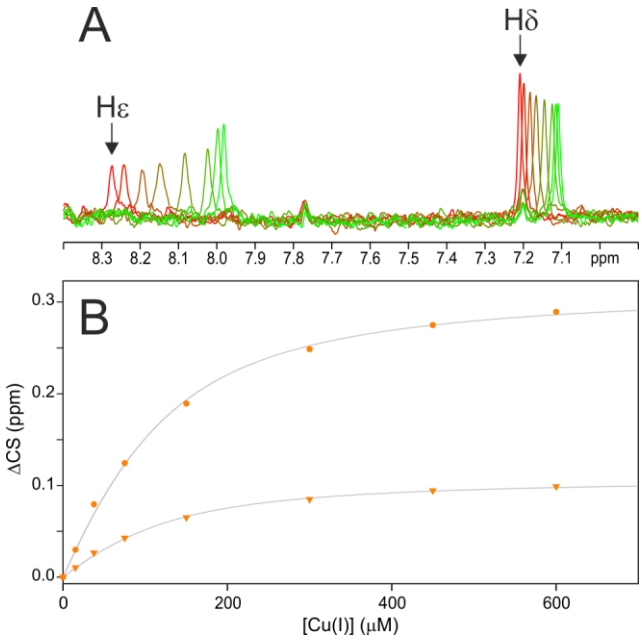

Figure S2. NMR analysis of Cu(I) binding to the 45-55 AS peptide. (A) 1D ^{1}H NMR spectra (6.9-8.4 ppm) of 45-55 AS peptide (75 μM) in the absence (red) and presence of increasing concentration (0-8 equivalents) of Cu(I) (green). The Hε and Hδ protons in the imidazole ring of His-50 are identified. (B) The panel shows the binding curves of Cu(I) to 45-55 AS peptide as monitored by changes in the chemical shifts ($\Delta\delta^{1}$H) of Hε (orange circle) and Hδ (orange triangle) protons of His-50. The apparent dissociation constant derived from this study (K_d = 55 ± 5μM) is consistent with that estimated for the His-50 site by 2D ^{1}H-^{15}N HSQC experiments. All experiments were recorded in D_2O, buffer MES 20 mM, NaCl 100 mM, pH 6.5 at 15ºC.

References

[S1] W. Hoyer, D. Cherny, V. Subramaniam, T.M. Jovin, Biochemistry 43 (2004) 16233–16242.

[S2] M.C. Miotto, E.E. Rodriguez, A.A. Valiente-Gabioud, V. Torres-Monserrat, A. Binolfi, L. Quintanar, et al., Inorg. Chem. 53 (2014) 4350–4358.

[S3] A. Binolfi, A.A. Valiente-Gabioud, R. Duran, M. Zweckstetter, C. Griesinger, C.O. Fernandez, J. Am. Chem. Soc. 133 (2011) 194–196.

[S4] P. Kuzmic, Anal. Biochem. 237 (1996) 260–273.

[S5] M. Rückert, G. Otting, J. Am. Chem. Soc. 122 (2000) 7793–7797.

[S6] A. Binolfi, R.M. Rasia, C.W. Bertoncini, M. Ceolin, M. Zweckstetter, C. Griesinger, et al., J. Am. Chem. Soc. 128 (2006) 9893–9901.

[S7] F. Delaglio, S. Grzesiek, G.W. Vuister, G. Zhu, J. Pfeifer, A.D. Bax, J. Biomol. NMR. 6 (1995) 277–293.