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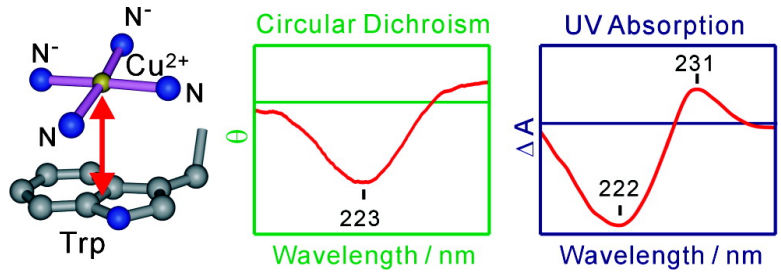
J. Am. Chem. Soc., **2008**, 130 (46), 15266-15267 • DOI: 10.1021/ja807010f • Publication Date (Web): 22 October 2008



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Published on Web 10/22/2008

Evidence for the Cation−π Interaction between Cu^{2+} and Tryptophan

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The cation−π interaction, an electrostatic attraction between a cation and a π electron system, is increasingly recognized as an important noncovalent force that influences the structures and functions of a variety of molecules including proteins and nucleic acids.[1,2] In proteins, a positively charged amino acid side chain or a metal cation interacts with the π electron system of an aromatic side chain of Phe, Tyr, or Trp.[2,3] Like other metal cations, the transition metal cation Cu^{2+}, which plays an important role in many proteins, is also expected to be involved in cation−π interactions. However, no experimental observation of such interaction has been reported so far, probably because of the oxidative and covalent-bond-forming reactivity of Cu^{2+} against the π electron system, in particular of the Trp indole ring.[2,4,5] Here we report the first spectral evidence for the cation−π interaction between Cu^{2+} and Trp in peptides. Our finding suggests that coordination of negatively charged ligands to the Cu^{2+} ion may decrease the reactivity of the cation to be suitable for the cation−π interaction. This study also proposes a new circular dichroism (CD) marker of the Cu^{2+}−Trp cation−π interaction.

Our discovery of the Cu^{2+}−Trp cation−π interaction has been initiated by measuring the CD spectrum of a 10-mer bioactive peptide, neuromedin C (NMC, Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met-NH_2; the last NH_2 stands for C-terminal amidation).[6] NMC has an amino-terminal Cu^{2+}/Ni^{2+} binding (ATCUN) motif ^{1}Xaa-^{2}Xaa-^{3}His, which coordinates to a Cu^{2+} or Ni^{2+} ion by using four nitrogen ligands: a free N-terminus nitrogen of the first residue (^{1}Xaa), two deprotonated main chain amide nitrogens of the second (^{2}Xaa) and third (^{3}His) residues, and an imidazole ring nitrogen of ^{3}His.[7]

CD spectra of NMC in the absence and presence of an equimolar Cu^{2+} are compared in Figure 1. The spectrum in the absence of Cu^{2+} is dominated by a strong negative band at 198 nm assignable to an irregular structure (orange line).[8] In the presence of equimolar Cu^{2+}, a new negative band with considerable intensity shows up at 223 nm (blue line). Since the main CD band at 198 nm does not change much, the newly produced 223-nm negative band may not be simply ascribed to a conformational change of the peptide.

To examine the aromatic contribution to the 223-nm negative CD band, we prepared a mutant W4L-NMC, in which the Trp residue at position 4 is replaced by Leu. As Figure 1 shows, the Cu^{2+} binding to W4L-NMC does not produce any distinct negative band around 223 nm (purple line). This observation strongly suggests that the 223-nm negative band of the NMC−Cu^{2+} complex arises from an interaction between Cu^{2+} and Trp.

The possibility of the Cu^{2+}−Trp interaction was further tested by using a trimer peptide representing the ATCUN motif (GNH, Gly-Asn-His-NH_2) and a tetramer peptide extending to the fourth residue Trp (GNHW, Gly-Asn-His-Trp-NH_2). GNHW exhibits

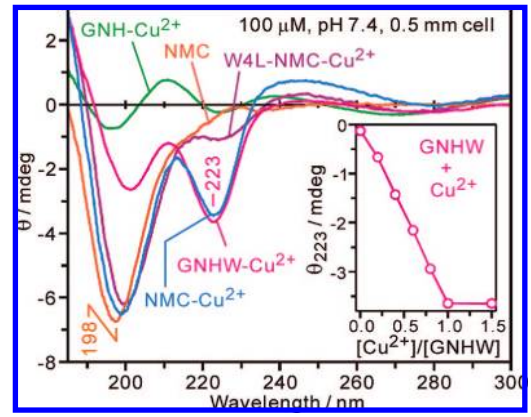

Figure 1. CD spectra of NMC, W4L-NMC, GNHW, and GNH (100 μM) in the presence of equimolar Cu^{2+} at pH 7.4 (1 mM HEPES buffer). For NMC, the spectrum in the absence of Cu^{2+} is also shown for comparison. The value of θ at 223 nm is plotted against the Cu^{2+}/peptide molar ratio for GNHW in the inset.

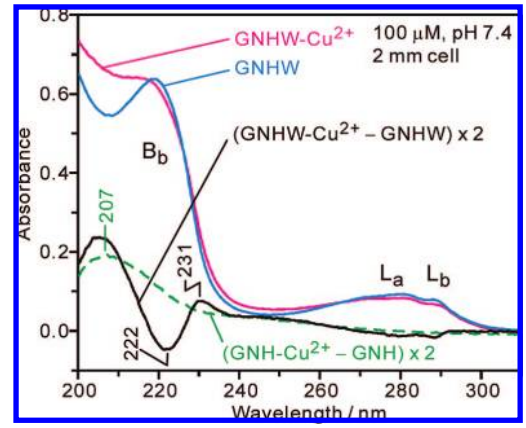

Figure 2. UV absorption spectra of GNHW (100 μM) and its 1:1 complex with Cu^{2+} at pH 7.4 (1 mM HEPES buffer). The difference spectrum GNHW−Cu^{2+} *minus* GNHW is doubly magnified for clarity. The broken line shows the corresponding difference spectrum for GNH−Cu^{2+}, indicating the effect of Cu^{2+} binding to the ATCUN motif region.

a negative CD band at 223 nm upon binding to a Cu^{2+} ion as in the case of NMC (red line in Figure 1), whereas GNH does not (green line). This observation confirms that the negative CD band at 223 nm is due to an interaction of the Trp residue with the Cu^{2+} ion bound to the ATCUN motif (GNH). The stoichiometry of the GNHW−Cu^{2+} complex is 1:1 as evidenced by the plot of the ellipticity at 223 nm against the metal/peptide molar ratio (see the inset of Figure 1).

The Cu^{2+}−Trp interaction also affects the UV absorption spectrum. Figure 2 shows the absorption spectra of GNHW in the absence and presence of equimolar Cu^{2+}, together with the

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10.1021/ja807010f CCC: $40.75

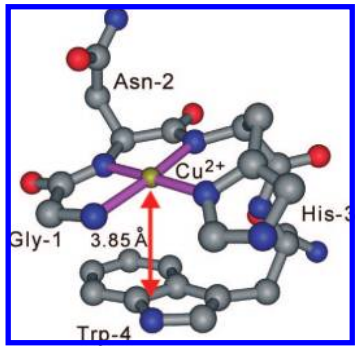

Figure 3. Model for the Cu^{2+}-Trp cation$-\pi$ interaction in the GNHW$-Cu^{2+}$ complex obtained by molecular mechanics calculations.

difference spectrum GNHW$-Cu^{2+}$ *minus* GNHW. To distinguish the effect of $Cu^{2+}-$Trp interaction from that of Cu^{2+} coordination by the ATCUN motif, the difference spectrum GNH$-Cu^{2+}$ *minus* GNH is also shown (green broken line). The Cu^{2+} coordination produces a broadband peaking around 207 nm, which is likely to be a $Cu^{2+}-$ligand charge transfer absorption.[9] Comparison of the difference spectrum of GNHW$-Cu^{2+}$ (black line) with that of GNH$-Cu^{2+}$ reveals a negative band at 222 nm and a weak positive band at 231 nm attributable to a $Cu^{2+}-$Trp interaction. This negative/positive band pair may reflect a weakening and a small red-shift of the strong B_b transition of the Trp indole ring.[10] An analogous UV difference spectrum with a negative/positive band pair around 220/230 nm has been observed for an indolyl model compound of the cation$-\pi$ interaction,[10] *N*,*N*′-bis(2-(3-indolyl) ethyl)-4,13-diaza-18-crown-6 (IE18C6), in which two indole rings are attached to a crown ether and they sandwich a K^+ ion trapped in the hole of the crown ring.[11] A cation$-\pi$ interaction between a positively charged His imidazole ring and a nearby Trp indole ring has also been reported to produce an analogous band pair.[10] The similarity of the UV difference spectrum of GNHW$-Cu^{2+}$ with those reported previously for other types of cation$-\pi$ interactions indicates that the $Cu^{2+}-$Trp interaction in GNHW$-Cu^{2+}$ is also categorized as a cation$-\pi$ interaction.

To gain insight into the geometry of the $Cu^{2+}-$Trp cation$-\pi$ interaction, the structure of the GNHW$-Cu^{2+}$ complex was investigated by molecular mechanics calculations using GROMACS software[12] and the OPLSAA force field[13] with a modification for the deprotonated amide groups in the ATCUN motif (see Supporting Information for details). Figure 3 shows the most stable structure obtained by the calculations. The Cu^{2+} ion bound by the four nitrogen ligands of the ATCUN motif is located at a distance of 3.85 Å from the Trp indole ring plane, and the cation lies on the five-membered ring when projected normal to the indole ring plane. Although the metal$-$indole distance is somewhat longer than that (3.31 Å) found in the IE18C6$-K^+$ complex,[11] the calculated structure of GNHW$-Cu^{2+}$ is consistent with the cation$-\pi$ interaction.

The structure of GNHW$-Cu^{2+}$ in Figure 3 bears a strong resemblance to that proposed for a Ni^{2+} complex of NMC on the basis of NMR data.[14] However, the distinct negative CD band at 223 nm observed for NMC$-Cu^{2+}$ and GNHW$-Cu^{2+}$ (Figure 1) is not seen for the Ni^{2+} complexes of these peptides (Supporting Information, Figure S1). Thus, the negative CD band at 223 nm must be characteristic of the $Cu^{2+}-$Trp cation$-\pi$ interaction, though the mechanism of the Cu^{2+}-specific CD activity is not clear at present. On the other hand, a negative/positive band pair around 220/230 nm in UV difference absorption is also seen for the GNHW$-Ni^{2+}$ complex (Supporting Information, Figure S2), being consistent with the previous finding that a weakening of the B_b absorption accompanied by a small red shift is common to cation$-\pi$ interactions involving the indole ring and does not depend on the kind of cation.[10] Recently, an analogous absorption change has also been reported for the cation$-\pi$ interaction between the monovalent Cu^+ and Trp.[15]

The prion protein, whose misfolding is implicated in the pathogenesis of prion diseases, contains four-tandem repeats of an octapeptide sequence PHGGGWGQ in its N-terminal region. The octapeptide repeats bind Cu^{2+} ions and give a strong negative CD band around 225 nm.[16] Studies by NMR[17] and molecular dynamics simulation[18] have proposed that the Trp side chain is in close proximity to Cu^{2+}. The 225-nm negative CD band reported for the prion protein may also be ascribed to a $Cu^{2+}-$Trp cation$-\pi$ interaction. Our previous Raman spectroscopic study revealed that Cu^{2+} binds to deprotonated main chain amide nitrogens of the octapeptide PHGGGWGQ as well as to one of the His imidazole nitrogens.[19] The coordination of deprotonated amide nitrogens to the Cu^{2+} ion also takes place in NMC$-Cu^{2+}$ and GNHW$-Cu^{2+}$ as described above. Accordingly, it is likely that the binding of deprotonated amides to Cu^{2+} is important for the cation to be involved in a cation$-\pi$ interaction. The oxidative reactivity of Cu^{2+} against the π electron system might be partly reduced by the ligation of negatively charged amide nitrogens, which are known to stabilize Cu^{2+} over Cu^+.[20]

Our finding of the $Cu^{2+}-$Trp cation$-\pi$ interaction and its signature in the CD spectrum may be useful in future studies on the role of Cu^{2+} in protein structure and function.

Acknowledgment. This work was supported in part by Grant-in-Aid for Scientific Research (B) (16350003) from the Japan Society for the Promotion of Science.

Supporting Information Available: Details of experimental and computational procedures. CD and UV absorption spectra of the Ni^{2+} complexes of NMC, GNHW, and GNH. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA807010F