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Tryptophan π-Electron System Capping a Copper(I) Binding Site—A New Organometallic Bonding Mode in Proteins

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Until recently, common textbook knowledge of organometallic compounds in biology was limited to enzymes dependent on cobalamin as cofactor. Their function relies on Co^{III} coordination to a carbon atom from methyl- or 5′-adenosyl. Other examples include the Ni–CO bond in the nitrogenase enzyme family[1,2] and the toxicity of carbon monoxide due to strong Fe–CO bonds in haem. Any speculation about other possible organometallic biochemistry would most probably refer to metal centres in enzymes. In contrast, two recent publications[3,4] describe Cu^{I} and Ag^{I} complexes of the prokaryotic transport protein CusF that show metal coordination to the π-electron density of the aromatic indole moiety of a Trp side chain. X-ray absorption and UV resonance Raman spectroscopy data verified the η^2-arene complex of Cu^{I} in the protein coordination framework observed through crystal structure analyses. Such a cation–π interaction observed in a metalloprotein constitutes a new organometallic coordination compound in biochemistry.

Copper is an essential trace element useful as an active site of redox-active enzymes in microbes, plants and mammals. The occurrence of Cu-binding proteins within the three domains of life was recently investigated by a bioinformatic approach.[5] The metabolism of living cells requires distinct transport systems for the uptake and efflux of Cu ions to maintain physiological concentrations.[6] A review on microbial and eukaryotic copper accumulation and mechanisms of distribution and regulation appeared very recently.[7]

Homeostatic regulation refers not simply to distinct Cu acquisition to equip Cu-dependent enzymes with their redox centres. As accumulation of redox-active metal ions is a potential origin of oxidative stress, regulatory cascades of proteins prevent the occurrence of simple aqua complexes of transitions metal ions in the cytosol of prokaryotic[8] and eukaryotic cells.[9] Specific transport proteins, so-called metallochaperones, are responsible for trafficking and binding the metal cofactors in the specific target proteins within the cell.[10] In the cytoplasm, copper chaperones coordinate Cu^{I} ions with Cys-thiolate side chains, whereas Met-rich motifs are preferred for Cu coordination[11] in potentially oxidising cellular environments for extracellular copper acquisition and Cu trafficking in the periplasmic space, probably to avoid oxidative reactions such as disulfide cross-links in the coordination site. Despite the different coordination characteristics of the sulfur ligands, both groups of copper chaperones coordinate Cu^{I} selectively and have accessible binding sites for transfer events. The specific features of thioether biochemistry in copper ion binding and trafficking were summarised recently.[12]

Bacteria have developed very efficient efflux mechanisms to decrease the concentration of potentially toxic compounds. These compounds induce the expression of protein complexes that are known as specific or multidrug efflux transporters. The defence mechanism of *E. coli* against potentially toxic silver and copper concentrations relies on the expression of proteins of the *cusCFBA* operon.[13,14] The proteins CusA, CusB and CusC establish an efflux system comparable to those of protein complexes known as the resistance nodulation division (RND) superfamily, which confer drug resistance in Gram-negative bacteria.[15] CusA is a proton-driven antiporter residing in the inner membrane, while CusC is an outer-membrane protein. Both span the periplasmic space connected by the membrane fusion protein CusB, which recently was characterised by EXAFS to coordinate a Cu^{I} or Ag^{I} ion threefold with methionine residues.[16] The periplasmic protein CusF is required for optimal function of the efflux system, interacting with CusB and CusC.[15] Homologues of CusF are observed in all putative silver/copper tolerance systems.[3]

This small protein (ca. 90 amino acid residues) is supposed to be a carrier for Cu^{I} or Ag^{I} ions. High-resolution crystal-structure analyses revealed a new and unexpected type of coordination of these metal ions in biological systems.[3,4] The structure of the metal-free *apo*-form of CusF was determined by X-ray crystallography.[17] The tertiary structure of CusF is a β-barrel, which is retained in the high-resolution crystal structures of its Ag^{I} and Cu^{I} complexes and verified by NMR spectroscopy in solution. Only minimal local changes of coordinating side chains occur upon metal coordination, as indicated by an overall rmsd of backbone atoms of about 0.5 Å (Figure 1).

At a first glance, the metal(I) ion is trigonally coordinated by the imidazole of His36 and the two thioethers from Met47 and Met49, but the metal is out of plane by 0.5 Å towards the indole moiety of Trp44; this completes a tetrahedral coordination shell as a η^2-ligand

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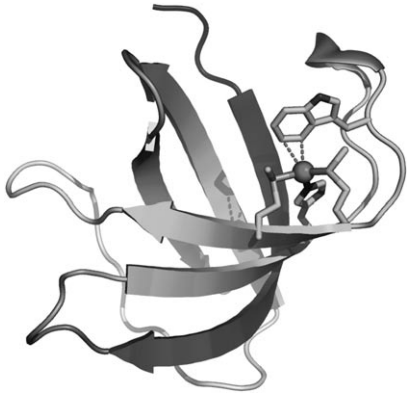

Figure 1. Tertiary structure of CusF showing a β-barrel of two orthogonally packed β-sheets, known as an OB fold.[4] The Cu^{I} ion is shown as a sphere with coordinating side chains His36, Met47, Met49 and Trp44 in stick representation. The η^2-coordination to Trp44 is indicated by dotted lines. Graphic created with PyMOL,[34] atomic coordinates from PDB ID: 2vb2.

(with Cε3 and Cζ3). The corresponding Cu–C (2.67 and 2.86 Å) and Ag–C lengths (2.99 and 3.29 Å) are at the long end of comparable small-molecule complexes in the Cambridge Structure Database. The radius of the higher homologue, Ag^{I}, is 38 pm larger than that of Cu^{I};[18] this easily explains the differences of 32 and 43 pm in the bond lengths towards the indole ring of Trp44.

The oxidation state of Cu^{I} and its high-resolution coordination geometry were confirmed by X-ray absorption spectroscopy.[3,4] These experiments already indicated the Cu–C interaction of the indole ring. O'Halloran's group further investigated the coordination by UV resonance Raman spectroscopy in solution,[4] which is a very sensitive tool for changes in the tryptophan environment.[19] The results are in agreement with a strong cation–tryptophan π-interaction for both metal complexes of CusF. Direct metal–carbon binding with π or π* systems has to be considered as covalently bound. In contrast, the important biochemical feature of the cation–π interaction in protein structure stabilisation, recognition and enzyme reaction pathways is an electrostatic noncovalent interaction.[20] Taking into account that the observed metal–carbon separations in the CusF complex are significantly longer than in comparable small-molecule complexes, the tryptophan interaction to Cu^{I} and Ag^{I} should be considered as an organometallic π-complex with η^2-arene coordination.

The binding of the four amino acid residues, and in particular the indole side chain of Trp44, to the copper(I) centre plays a pivotal role in the biological function of the transport protein CusF. It therefore warrants closer inspection. The binding pocket for the metal centre, Cu^{I} or Ag^{I}, consists of the amino acids His36, Met47, Met49 and the capping ligand Trp44. The sulfur atoms of the two methionine groups and the imidazole nitrogen atom of His36 form σ-donor bonds to copper(I) or silver(I), whereas Trp44 acts as π-donor ligand towards the metal.

It might be beneficial to briefly describe the bonding in general terms. Both, σ- and π-donor bonds are common in organometallic chemistry,[21] the σ-donor bonds being significantly stronger. Copper(I) is a d^{10} closed-shell metal ion and isoelectronic to nickel(0). Like Ni^{0}, Cu^{I} prefers a tetrahedral coordination geometry, although tricoordinate compounds are well known.[22,23] It is easily oxidised to Cu^{II} due to Jahn–Teller stabilisation in the ensuing d^{9} metal ion. Copper(II) has a distorted tetrahedral coordination sphere, similar to that of Cu^{I}. The situation for Ag^{I} is different in as much as it prefers a linear coordination geometry, but many examples of T-shaped and tetrahedral geometries are known.[24]

Among various reported small-molecule structures of Cu^{I} complexes with π-donor ligands is an example of η^2-coordination to an indole ring.[25] In this case, the coordinating carbon atoms belong to the five-membered ring and are equidistant from the Cu^{I} centre, see Figure 2A.

In medicinal chemistry, ruthenium centres are used for diagnostic purposes due to specific π-bonding to the aromatic Phe side chain even in the presence of indole and imidazole groups from Trp and His residues.[26–28]

Conry et al. have developed a copper(I) model system based on the tridentate S,S,N-macrocyclic ligand 1-aza-4,8-dithiacyclodecane.[29–32] Like its bioinorganic analogue, this tridentate ligand binds with two thioether and one tertiary amine entities in S,S,N. This flat trigonal pyramidal geometry can be completed to a distorted tetrahedron by either an external ligand (acetonitrile, triphenylphosphane PPh_3) or an intramolecular ligand such as a pendant naphthene ring, which forms a η^2 arene complex of Cu^{I}, see Figure 2B.[30] Although this was not mentioned by the authors, it can reasonably be assumed that the phosphane ligand is not easily removed from

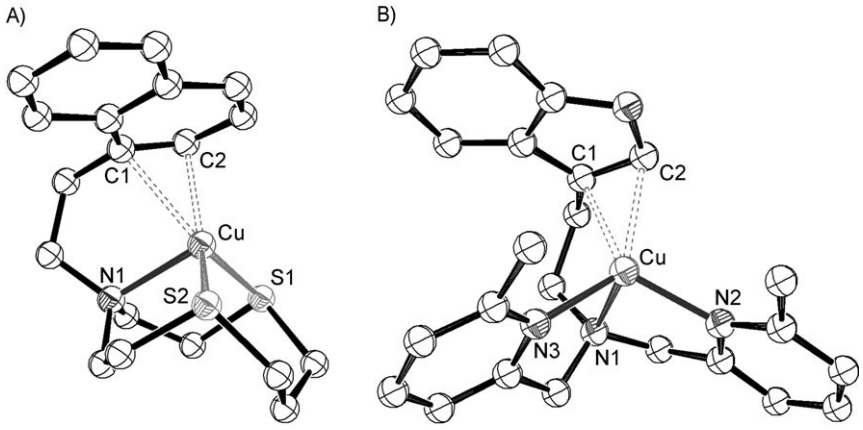

Figure 2. Examples of η^2-arene coordination of small-molecule cationic Cu^{I} compounds. Graphics created with ORTEP-3 at the 30% probability level.[35] A) Crystal structure of the cationic complex of Cu^{I} with tridentate *N*-(3-indolylethyl)-*N*,*N*-bis(6-methyl-2-pyridylmethyl)amine showing the Cu^{I}–η^2-indole coordination.[25] The Cu–C bond lengths are almost equal (2.23 and 2.27 Å). B) Crystal structure of the η^2-naphthyl-1-aza-2,4,8-dithiacyclodecane Cu^{I} complex.[31] Two thioether sulfurs and the tertiary amine nitrogen coordinate as common σ-donor ligands to Cu^{I}, and the tetrahedral sphere is completed by the π-electron system of the naphthyl ring. The shorter Cu^{I}–C bond (2.13 Å) is similar to the Cu–N bond (2.15 Å). The other Cu–C bond length is about 2.3 to 2.4 Å in two different crystal structures. The Cu–S bond lengths are around 2.25 Å.

the copper binding site. This is in stark contrast to the somewhat dynamic behaviour of the naphthalene and acetonitrile ligands. Of course, PPh_3 is a strong σ-donor and poor π-acceptor ligand; whereas acetonitrile is a weak σ-donor, and the delocalised π-electron system of naphthalene is a weak π-donor ligand. Thus, acetonitrile and the pendant naphthyl ring are seen to compete for the fourth coordination site on copper(I). Actual coordination is dependant on the reaction conditions, in particular the CH_3CN concentration. At low CH_3CN concentration, the chelate effect results in naphthalene coordination whereas with increasing acetonitrile concentration, the weak σ-donor ligand evicts the weak π-donor ligand.

Solid-state structures of small-model complexes are not always preserved in solution,[22,23] but an elaborate 2D $^1H,^{13}C$ NMR study of the model Cu^I complex with a hemilabile naphthyl ligand proved invaluable in showing that the solid-state structure is essentially preserved in solution. The η^2-naphthyl ligand indeed shows dynamic behaviour on the NMR timescale and binds with 12–13 kcal mol^{-1}.[29] This type of weak binding supports metal ion exchange between proteins in the specific transport systems.

It is worth mentioning that reaction of the potentially tridentate S,S,N ligand with Cu^{II}bromide results in S,S coordination with a pendant amine functionality as the two coordinating bromide anions occupy two binding sites on the copper ion. The copper(II) complex features the expected distorted tetrahedral coordination geometry similar to that seen in other copper(I) complexes and in Cu^I–CusF. But the discussed S,S,N, π coordination site of CusF binds specifically Cu^I or Ag^I, showing weak affinity for Cu^{II} ions. In contrast, CusF is known to coordinate Cu^{II} ions with histidine residues of the N-terminal part of the protein.[33] This part of the polypeptide was truncated for crystallography and spectroscopy of both metal(I) complexes.

Having seen that a σ-donor ligand usually binds more strongly to the metal centre than a π-donor ligand, we can now ask ourselves why the Trp44 residue binds with one of the C=C double bonds in π-donor mode rather than with the presumably stronger σ-donor nitrogen site. The answer, as so often in coordination chemistry, is geometric constraints. The four amino acid binding sites His36, Met47, Met49 and Trp44 are embedded in the overall protein structure and have only limited flexibility. In order to bind with the σ-donor nitrogen site, the Trp44 residue would have to rotate by some 90°, thus effectively bringing the nitrogen atom into the copper position. Binding of Cu^I would be impossible in this event.

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