DNA-Dependent Cu(II)•Xaa-Xaa-His Metal-Peptide Dissociation

Mark A. Lewis[1], Kristie D. Goodwin[2], Millie M. Georgiadis[2], and Eric C. Long[1]

[1]Department of Chemistry & Chemical Biology, Purdue School of Science and [2]Department of Biochemistry & Molecular Biology, Indiana University School of Medicine Indiana University-Purdue University Indianapolis (IUPUI)

Metallopeptides of the general form M(II)•Xaa-Xaa-His (where M is Cu or Ni) are employed as affinity cleavage and activity-modifying appendages to DNA binding motifs and occur naturally at the amino-terminus of human protamine P2A (Arg-Thr-His). In our own work, we have documented the ability of such metallotripeptides alone to interact selectively with the DNA minor groove and mediate the strand scission of A/T-rich regions, and other sites, via C4´-H abstraction.

In a more recent effort to examine the DNA site-selectivity exhibited by combinatorial libraries of M(II)•Xaa-Xaa-His metallopeptides, we have applied the high-throughput fluorescent intercalator displacement (HT-FID) assay developed by Boger *et al*. During the course of these experiments, we have observed that Cu^{2+} and Ni^{2+} are released from M(II)•Xaa-Xaa-His metallopeptides in a DNA-dependent fashion leading to the presence of some free M^{2+} and apo-peptide despite the relatively high affinity displayed by Cu^{2+} or Ni^{2+} for Xaa-Xaa-His peptides in the absence of DNA ($K_D \sim 10^{-17}$-10^{-16} M); free M^{2+} then alters the fluorescence of the FID system in a DNA sequence-selective fashion. Metal-peptide dissociation was observed also to occur as a function of metallopeptide-DNA binding affinity; metallopeptides without the ability to interact significantly with DNA (*e.g*., charge-neutral Cu(II)•Gly-Gly-His-$CONH_2$) do not dissociate, while metallopeptides that bind to DNA dissociate readily (*e.g*., positively-charged Cu(II)•Arg-Gly-His-$CONH_2$). Further, X-ray crystallographic analysis of Cu(II)•Lys-Thr-His-$CONH_2$ + an oligonucleotide containing a 5´-ACCC site, a sequence that consistently indicates the release of free Cu^{2+} in the FID assay, revealed a surprisingly specific interaction between the apo-peptide and the DNA *major* groove. In this structure: (1) the His imidazole is coplanar with the G•C base pair 3´ to the A residue and hydrogen bonded to the N-7 of G; (2) the peptide carboxy-terminal amide interacts with the O-6 atom of this same G nucleobase; and (3) the remaining peptide (Lys-Thr-) is disordered and extends away from the DNA helix; Cu^{2+} is not observed in this structure.

While the above findings do not alter the conclusions of earlier DNA cleavage studies (that require a Ni(II)-bound peptide for activity), they do suggest that affinity of a M(II)•Xaa-Xaa-His metallopeptide for DNA, resulting in intimate contact between the two, can lead to metal release and perhaps "delivery" of an apo-peptide to the DNA; peptide alone in the FID assay does not alter the fluorescence of the system nor has evidence for a direct interaction between Lys-Thr-His (in the absence of a metal-bound intermediate) and 5´-ACCC been revealed to date. As will be discussed, these findings suggest a potential role for transition metal ions and DNA in the "delivery" and ultimate interaction of low MW agents, and perhaps proteins like protamine, with DNA.