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Spectroscopic Evidence for the Formation of Goldfingers

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Gold(I) has long been used in the treatment of rheumatoid arthritis, but the therapeutically relevant biological targets of gold(I) are not well understood. Here, we report the results of a spectroscopic investigation into the formation of goldfingers. By exploiting a thiolate to gold charge-transfer band in the UV, we observed that gold(I) interacts with zinc finger peptides with a stoichiometry of one gold ion for each two cysteine residues, forming 1:1, 1.5:1, and 2:1 adducts with zinc finger peptides containing CCHH, CCHC, and CCCC donor sets, respectively. In addition, circular dichroism experiments provided evidence that goldfingers are more ordered than the corresponding metal-free peptides but do not exhibit the canonical zinc finger structure.

Gold(I) complexes have been used in the treatment of rheumatoid arthritis for over 80 years,[1] but their mechanism(s) of action are still only poorly understood.[2–5] Gold(I) is a soft, thiophilic metal ion that readily undergoes ligand exchange to interact with biological thiolates under physiological conditions.[4,6–8] As an example, serum albumin, an abundant transport protein known to carry metal ions and other drugs throughout the body, coordinates to gold(I) through the cysteine residue at position 34.[9,10] The ability of gold to inhibit thiolate-dependent enzymes has been demonstrated as well.[11–22] The crystal structures of several gold(I)–enzyme adducts have been solved, indicating that gold(I) binds to the active-site cysteine residues of these enzymes and exerts its inhibitory effect by directly blocking the activity of the enzymes.[23,24] The crystal structure of gold(I) bound to human glutathione reductase provides an illustrative example.[23] Gold(I) is bound in a linear, two-coordinate geometry to cysteine 58 and cysteine 63 from the enzyme active site. Although interactions of gold(I) with thiolate-dependent enzymes can account for many of the biological effects of the gold(I) drugs, it is likely that there are other biological targets of gold(I) as well.

The biological effects of gold(I) are consistent with the decreased transcription of several genes, and there is growing evidence that the anti-inflammatory effects of gold(I) may be mediated through interactions with transcription factors.[25] Gold(I) has been shown to interfere with the activity of both metal-independent transcription factors and metal-responsive transcription factors. In addition, gold(I) has been shown to inhibit DNA binding of zinc finger proteins through gel-mobility shift assays.[26,27] The zinc finger family of transcription factors employs a tetrahedrally coordinated zinc ion to

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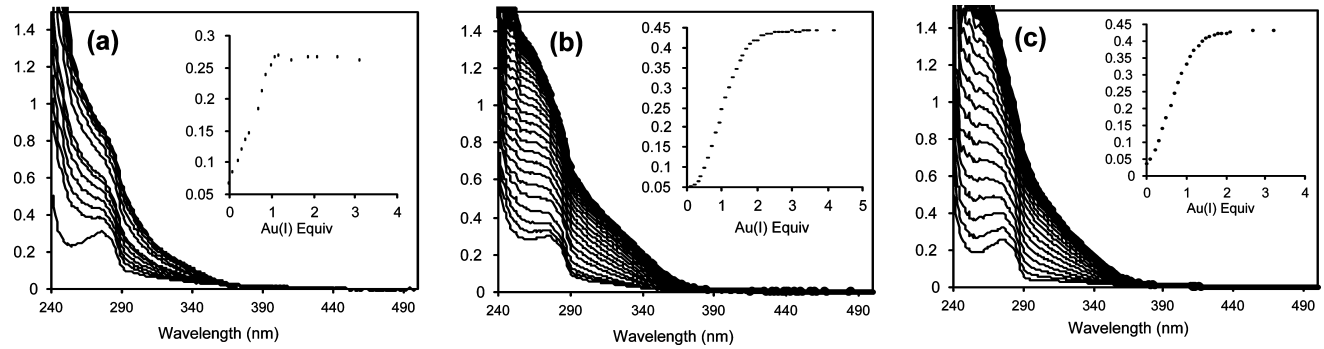
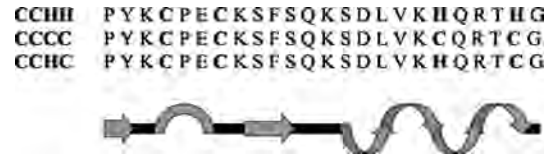


Figure 1. Titrations of reduced zinc finger peptides with Et_3P–Au–Cl monitored by UV/vis. The insets show increases in A_{310} as a function of added gold(I): (a) a solution of 1.34×10^{-4} M CCHH coordinates 1 mol equiv of gold(I); (b) CCCC (1.42×10^{-4} M) and (c) CCHC (1.50×10^{-4} M) bind 2 and 1.5 mol equiv of gold(I), respectively.

achieve the secondary structure necessary for DNA binding.[28–32] The zinc binding site in canonical zinc fingers has two cysteine-derived thiolate ligands and two histidine-derived nitrogen ligands coordinated to the metal ion (a CCHH zinc finger).[33] Biologically relevant variations of this sequence include CCHC and CCCC.[33,34] Recently, gold(I) has been shown to coordinate to a CCHH zinc finger peptide and to inhibit the formation of a zinc finger–DNA complex.[27] In addition, electrospray ionization mass spectrometry experiments indicate that gold(I) has a stronger affinity for this zinc finger than zinc(II) ($K_d^{Zn}/K_d^{Au} = 4.2$).[27] On the basis of these studies, it has been proposed that gold(I) could replace zinc(II) in zinc finger proteins at physiologically relevant concentrations. Because of the thiophilicity of gold(I) as well as its tendency to form two-coordinate, linear complexes, it is reasonable to propose that the gold(I) ion would coordinate to the two cysteine residues in the CCHH zinc finger, distorting the canonical zinc finger secondary structure and abrogating DNA binding activity. zinc fingers with CCHC and CCCC coordination sites could also presumably bind gold(I), although these interactions had not been studied. In order to better understand the gold(I)–zinc finger interaction, we undertook a spectroscopic investigation into the stoichiometry and structure of the gold(I)–zinc finger complexes. A 26-amino acid consensus zinc finger sequence has been identified as a model for metal binding to the full-length transcription factors and is often used in studies of metal binding and the effect of metal ions on zinc finger secondary structure.[33] The consensus sequences for the CCHH, CCHC, and CCCC zinc fingers are shown in Scheme 1.

One of the major challenges in the study of gold(I)-biomolecule adducts is the "spectroscopically quiet" d^{10} electron configuration of the ion. However, absorbances attributable to ligand to metal charge transfer bands have been identified at short wavelengths for several d^{10} metal ions, including Au(I), bound to cysteine residues in proteins.[35–38] The formation of Au(I)-thiolate complexes can be followed by monitoring the formation of a shoulder at 310 nm. In order to follow the formation of goldfingers, we chose to use Et_3P–Au–Cl, a known metabolite of auranofin that does not contain a thiolate ligand.[2] The phosphine-based reductant tris(2-carboxyethyl)phosphine (TCEP) was used to maintain the fully reduced state of the cysteine residues in the peptides without the addition of exogenous thiolates that would interfere with the electronic spectra.

Scheme 1[a]



[a] Sequences of the three zinc finger model peptides CCHH, CCCC, and CCHC along with a schematic indicates the secondary structure in the canonical zinc fingers where a straight arrow indicates β sheet, a curved arrow indicates α helix, and the curved line indicates a flexible loop.

As illustrated in Figure 1, Et_3P–Au–Cl binds readily to the zinc finger peptides that have been reduced with excess TCEP. As shown in Figure 1a, the absorbance of the Au(I)–CCHH mixture increased until a 1:1 molar ratio was reached, after which further additions of Et_3P–Au–Cl resulted in no subsequent increase in absorbance. The observation that 1 equiv of gold(I) can bind to each 1 equiv of CCHH is consistent with the data in the literature.[27] Previous investigations of metal binding to CCCC and CCHC zinc fingers invariably show that metals coordinate to this peptide model in a 1:1 ratio, as seen with CCHH.[33,34,37,39,40] The titration of the reduced CCCC peptide with Et_3P–Au–Cl is shown in Figure 1b. In this case, a 2:1 Au(I)/CCCC ratio was observed, independent of the peptide concentration, TCEP concentra-

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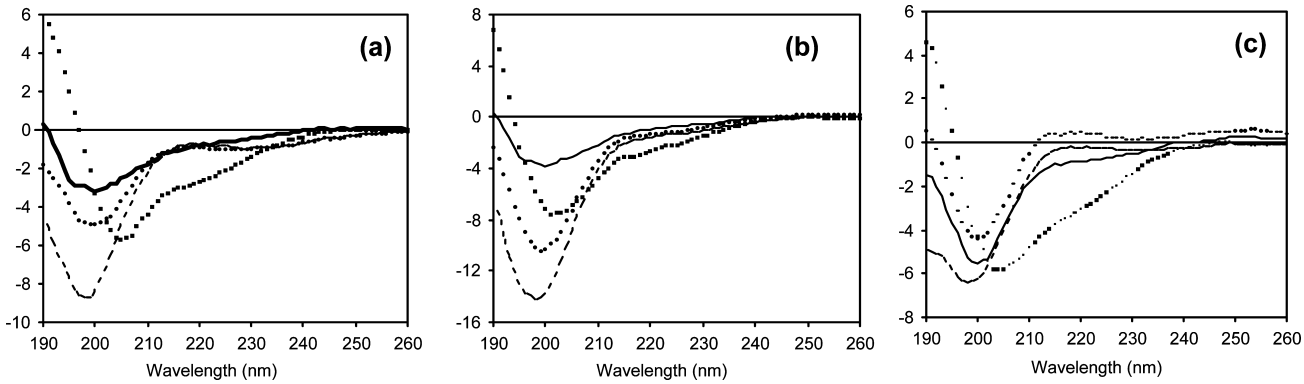

Figure 2. CD spectra of zinc fingers (a) CCHH, (b) CCCC, and (c) CCHC. The data represent zinc fingers (■), goldfingers (solid lines), reduced peptides (dashed lines), and oxidized peptides (●).

tion, and titration increment. To our knowledge, this is the first example of multiple metal ions binding to one zinc finger peptide. Previous studies of the metal-binding properties of CCCC have focused on metal ions capable of adopting a four-coordinate ligand environment. Although three- and four-coordinate gold(I) complexes are known, linear complexes are strongly preferred, especially in the presence of anionic ligands such as thiolates.[4] The titration of reduced CCHC with Et_3P–Au–Cl is shown in Figure 1c. The binding curve indicates that gold(I) coordinates to CCHC in a noninteger ratio. The absorbance at 310 nm increases until approximately 1.5 equiv of Et_3P–Au–Cl has been added. One possible explanation for the observation that two peptides seem to bind three gold(I) ions is the formation of two intramolecular Cys–Au–Cys centers and one intermolecular Cys_A–Au–Cys_B center. This type of coordination has been observed in metal-binding studies of flexible peptide models.[41]

It is well-known that zinc finger model peptides have no defined secondary structure if there is no metal present.[27,40] Circular dichroism (CD) spectra of the reduced and oxidized peptides alone and in the presence of zinc(II) and gold(I) are shown in Figure 2. The reduced (unstructured) zinc fingers have pronounced minima near 200 nm. The oxidized peptides are slightly more rigid because of the formation of disulfide bond(s) but also have distinct minima near 200 nm and little secondary structure. As expected, the zinc(II)–peptide adducts have strong maxima located at approximately 190 nm and broad minima at 205 nm.[40] These trends are consistent for all three of the zinc finger peptide motifs investigated.

When the reduced peptides are treated with Et_3P–Au–Cl, distinct spectra are obtained. The maximum at 190 nm, which corresponds to the formation of an α-helical secondary structure, is decreased significantly as compared to the parent zinc finger. It is clear that the α helix is compromised upon gold binding. In addition, the minimum seen at 205 nm in the intact zinc finger peptides is blue-shifted by approximately 5 nm upon gold(I) coordination. On the basis of these data, we conclude that the goldfingers have different secondary structures than their zinc finger counterparts. This observation correlates well with the biological data, indicating that goldfingers are no longer able to bind to DNA.[27] Again, these trends are consistent for peptides with a CCHH metal-binding motif, a CCHC motif, and a CCCC motif. It is important to note that the goldfingers, although they have less secondary structure than the corresponding zinc fingers, are more ordered than the metal-free peptides in either the oxidized or reduced state. In addition, no change is seen when an oxidized peptide is exposed to gold(I), providing further evidence that the thiol ligands must be available in order for gold to bind.

The secondary structure of zinc finger transcription factors is integral to their ability to recognize and bind DNA. As described above, the secondary structure of the goldfinger peptides is significantly different compared to the parent zinc fingers. The prominent difference is the lack of α-helical character, as evidenced by loss of the maximum at 190 nm in the CD spectra. Our data suggest that gold(I) could interfere with the DNA binding activity of zinc finger peptides by coordinating to the cysteine residues in the zinc binding site and inducing a conformational change.

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Supporting Information Available: Experimental details, electronic spectra of Et_3P–Au–Cl and TCEP, and titrations of TCEP and oxidized TCEP with Et_3P–Au–Cl. This material is available free of charge via the Internet at http://pubs.acs.org.

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