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Anna Fedorova

Michael Y. Ogawa

Bowling Green State University, [mogawa@bgsu.edu](mailto:mogawa@bgsu.edu)

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# Site-Specific Modification of de Novo Designed Coiled-Coil Polypeptides with Inorganic Redox Complexes

Anna Fedorova and Michael Y. Ogawa\*

Department of Chemistry and Center for Photochemical Sciences, Bowling Green State University, Bowling Green, Ohio 43403. Received August 9, 2001; Revised Manuscript Received October 26, 2001

A stepwise procedure for preparing of site-specific binuclear metalloproteins is described. The modification procedure involves the alkylation of a cysteine side chain by reaction with  $[\text{Ru}(\text{bpy})_2(\text{phen-CIA})]^{2+}$ , where  $\text{bpy} = 2,2'$ -bipyridine and  $\text{phen-CIA} = 5$ -chloroacetamido-1,10-phenanthroline, followed by the coordination of a ruthenium pentammine complex to a histidine residue located elsewhere along the sequence. The apo and metalated versions of the peptides C10H21(30-mer) and H10C21(30-mer) display circular dichroism spectra having minima at 208 and 222 nm, with  $\theta_{222}/\theta_{208} = 1.04$  to indicate that these peptides exist as  $\alpha$ -helical coiled-coils in aqueous solution. When the ruthenium polypyridyl complex is attached to C10H21(30-mer), the  $\Delta$ -I and  $\Lambda$ -I diastereomers of the resulting metalloprotein can be readily separated from each other by reversed-phase HPLC. However, in the case of the related H10C21(30-mer) metalloprotein, the two diastereomers cannot be chromatographically resolved. These results indicate how the subtle interplay between peptide conformation/sequence and metal complex geometry may alter some of the physical characteristics of metalloproteins.

## INTRODUCTION

The incorporation of extrinsic metal centers into proteins and polypeptides has facilitated the study of biological electron-transfer mechanisms (1, 2) and, more recently, the de novo design of minimalistic metalloprotein structures (3–9). Such procedures commonly involve either metal coordination to natural amino acid side chains such as histidine (4, 8, 10, 11) or cysteine (6, 12–14) or the use of non-natural amino acids that contain metal binding sites (15, 16). Our group has used previously developed histidine coordination methods to prepare a new series of synthetic metalloproteins (3, 5) in which the self-assembled  $\alpha$ -helical coiled-coil motif (17) is employed as a well-defined protein scaffold upon which metal-based electron donors and acceptors can be attached. Our studies have demonstrated that such systems can undergo either long-range, intraprotein electron-transfer across a well-defined, yet noncovalent, peptide-peptide interface (5) or inter-protein electron transfers whose rates can be modulated by electrostatic protein-protein interactions (3). In both these cases, the electron-transfer event involved inorganic cofactors that were placed on separate peptide strands of the  $\alpha$ -helical coiled-coil. In other situations, it may be desirable to place such cofactors at different positions along the backbone of a single peptide chain. However, the preparation of such peptides presents a special synthetic challenge as coordinatively unsaturated metal complexes can react promiscuously with a variety of potential ligands. Thus, the reaction of inorganic reagents with polypeptides often yields a mixture of difficult-to-separate substitutional isomers. The situation becomes even more complicated when two different metal complexes are to be bound to specific positions along the sequence of a given protein or polypeptide (18). In this paper, we present a straight-

forward solution to this problem in which a thiol-specific metalation reaction is used to attach a coordinatively saturated ruthenium polypyridyl group to a cysteine residue, followed by the coordination of a ruthenium pentammine site to a histidine residue located elsewhere along the sequence. Details of the synthesis, purification, and characterization of these peptides are described here, and their electron-transfer properties will be discussed elsewhere (19). It is also reported that the specific placement of a chiral ruthenium polypyridyl complex within the peptide can significantly alter the way the peptide interacts with solid chromatographic supports: one substitutional isomer of the ruthenium polypyridyl-containing metalloproteins,  $[(\text{Ru-phen})\text{-C10}]\text{H21}$ (30-mer), can be resolved into its two diastereomers by reversed-phase HPLC, whereas the other,  $\text{H10}[(\text{Ru-phen})\text{-C21}]\text{(30-mer)}$ , cannot. These results indicate that the derivatization of polypeptides with inorganic complexes can subtly alter the physical properties of the macromolecule.

## EXPERIMENTAL SECTION

**General Methods.** UV-vis, electrochemical, and MALDI-TOF mass spectral measurements were performed as previously described (5). Electrospray ionization mass spectra (ESI-MS) were obtained on a Bruker Daltonics Esquire LC-MS at the Department of Chemistry of the University of Toledo, Toledo, OH.

All peptide metalation reactions were monitored by HPLC on a semipreparative reversed-phase  $\text{C}_{18}$  column (Zorbax 300SB- $\text{C}_{18}$ ,  $9.4 \times 250$  mm,  $5 \mu\text{m}$  particle size,  $300 \text{ \AA}$  pore size) with an AB gradient of 1% B/min for 20 min followed by a gradient of 0.5% B/min. For both gradients, the flow rate was 2 mL/min and solvent A was 0.1% (v/v) HTFA in water and solvent B was 0.1% (v/v) HTFA in acetonitrile. Purification of bulk quantities of the peptides was achieved using a preparative  $\text{C}_{18}$  column

\* To whom correspondence should be addressed. Phone: (419) 372-2809. Fax: (419) 372-9809. E-mail: mogawa@bgnet.bgsu.edu.

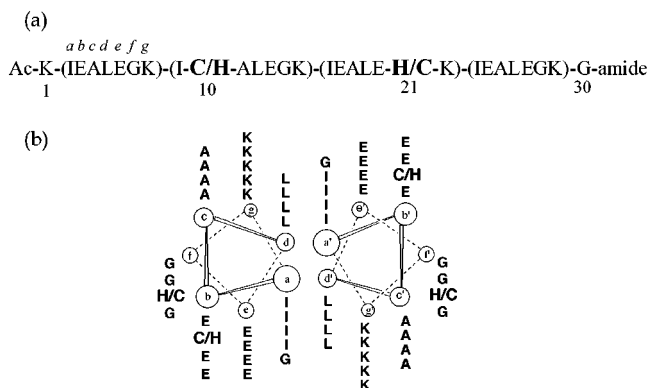
(Zorbax 300SB-C<sub>18</sub>, 21.1 mm × 25 cm, 7 μm particle size, 300 Å pore size) using the same gradient as described above but at a flow rate of 5 mL/min.

**Peptide Synthesis.** Solid-phase techniques were used to prepare the 30-residue polypeptides, C10H21(30-mer): Ac-K-(IEALEGK)(ICALEGK)(IEALEHK)(IEALEGK)-G-amide, and H10C21(30-mer): Ac-K-(IEALEGK)(HIALEGK)-(IEALECK)(IEALEGK)-G-amide, on an Applied Biosystems model 433A peptide as previously described (5). ESI-MS *m/z* (ion): found for C10H21(30-mer) 1088.2 ([M + 3H<sup>+</sup>]<sup>3+</sup>), 816.4 ([M + 4H<sup>+</sup>]<sup>4+</sup>) 653.4 ([M + 5H<sup>+</sup>]<sup>5+</sup>), 544.6 ([M + 6H<sup>+</sup>]<sup>6+</sup>); for H10C21(30-mer) 1088.1 ([M + 3H<sup>+</sup>]<sup>3+</sup>), 816.5 ([M + 4H<sup>+</sup>]<sup>4+</sup>), 653.3 ([M + 5H<sup>+</sup>]<sup>5+</sup>), 544.6 ([M + 6H<sup>+</sup>]<sup>6+</sup>).

**Synthesis of [Ru(bpy)<sub>2</sub>(phen-CIA)](PF<sub>6</sub>)<sub>2</sub>.** *N,N*-Dicyclohexylcarbodiimide (DCC) (2.5 g, 1.2 mmol) was added to a solution of chloroacetic acid (2.55 g, 2.7 mmol) dissolved in anhydrous ethyl acetate (75 mL). A white precipitate was seen to form immediately, and the mixture was stirred at ambient temperature for 2 h. The reaction mixture was then filtered, and the volume of the filtrate was reduced by rotary evaporation to yield chloroacetic anhydride as a viscous yellow oil. The oil was dissolved in acetonitrile (30 mL) and added to a solution of [Ru(bpy)<sub>2</sub>(phen-NH<sub>2</sub>)](PF<sub>6</sub>)<sub>2</sub> (2 g, 2 mmol) in acetonitrile (100 mL), where bpy = 2,2'-bipyridine and phen-NH<sub>2</sub> = 5-amino-1,10-phenanthroline. The mixture was stirred overnight, and the acetonitrile was then removed by rotary evaporation. The resulting solid was redissolved in a minimal amount of methanol and precipitated with the addition of saturated NH<sub>4</sub>PF<sub>6</sub> (aq). The Ru(bpy)<sub>2</sub>(phen-CIA)(PF<sub>6</sub>)<sub>2</sub>, where phen-CIA = 5-chloroacetamido-1,10-phenanthroline, was collected by vacuum filtration as an orange solid, washed with water followed by diethyl ether, and stored at room temperature in the dark. ESI-MS *m/z* (ion): 830.3 ([M - PF<sub>6</sub><sup>-</sup>]<sup>+</sup>), 684.3 ([M - 2PF<sub>6</sub><sup>-</sup> + e<sup>-</sup>]<sup>+</sup>).

**Synthesis of [Ru(bpy)<sub>2</sub>(phen-IA)](PF<sub>6</sub>)<sub>2</sub>.** This synthesis was performed as outlined above for [Ru(bpy)<sub>2</sub>(phen-CIA)](PF<sub>6</sub>)<sub>2</sub> except iodoacetic acid was used in place of chloroacetic acid. ESI-MS *m/z* (ion): 922.1 ([M - PF<sub>6</sub><sup>-</sup>]<sup>+</sup>), 776.2 ([M - 2PF<sub>6</sub><sup>-</sup> + e<sup>-</sup>]<sup>+</sup>).

**Peptide Labeling with [Ru(bpy)<sub>2</sub>(phen-CIA)](PF<sub>6</sub>)<sub>2</sub>.** In a typical procedure, a sample of either C10H21(30-mer) or H10C21(30-mer) (5 mg, 1.28 μmol) was dissolved in 100 mM phosphate buffer (1 mL, pH 7.0), and the resulting solution was purged with argon gas for 15–20 min. To this was added solid tris(2-carboxyethyl)phosphine (ca. 5 mg, 0.017 mmol). After being stirred under an argon atmosphere for ca. 20 min, the solution was neutralized by the dropwise addition of 1 N NaOH (aq). A solution of [Ru(bpy)<sub>2</sub>(phen-CIA)](PF<sub>6</sub>)<sub>2</sub> (10 mg, 0.01 mmol) dissolved in *N,N*-dimethylformamide (ca. 0.5 mL) was then added to the reaction mixture. After the mixture was stirred for 4–5 h at room temperature, it was applied directly onto a size-exclusion column (Biogel P-2) that was previously equilibrated with 0.1 N trifluoroacetic acid (HTFA). Elution with the HTFA solution yielded the peptide fraction first followed by the hydrolyzed metal complex. The collected peptide fraction was then analyzed by HPLC and in cases where unreacted apo-peptide was detected, the metalation procedure was repeated using a fresh sample of [Ru(bpy)<sub>2</sub>(phen-CIA)](PF<sub>6</sub>)<sub>2</sub>. ESI-MS *m/z* (ion): found for [(Δ-*l*)(Ru-phen)-C10]H21(30-mer) 978. 2 ([M + 4H<sup>+</sup>]<sup>4+</sup>), 782.6 ([M + 5H<sup>+</sup>]<sup>5+</sup>), 652.4 ([M + 6H<sup>+</sup>]<sup>6+</sup>), 559.3 ([M + 7H<sup>+</sup>]<sup>7+</sup>); for [(Δ-*l*)(Ru-phen)-C10]H21(30-mer) 978. 1 ([M + 4H<sup>+</sup>]<sup>4+</sup>), 782.7 ([M + 5H<sup>+</sup>]<sup>5+</sup>), 652.5 ([M + 6H<sup>+</sup>]<sup>6+</sup>), 559.6 ([M + 7H<sup>+</sup>]<sup>7+</sup>); for H10[(Δ-*l* + Λ-*l*)(Ru(phen)-C21)](30-mer) 978.



**Figure 1.** (a) Differences in the primary sequence of the C10H21(30-mer) and H10C21(30-mer) peptides. (b) Helical wheel diagram of the peptides.

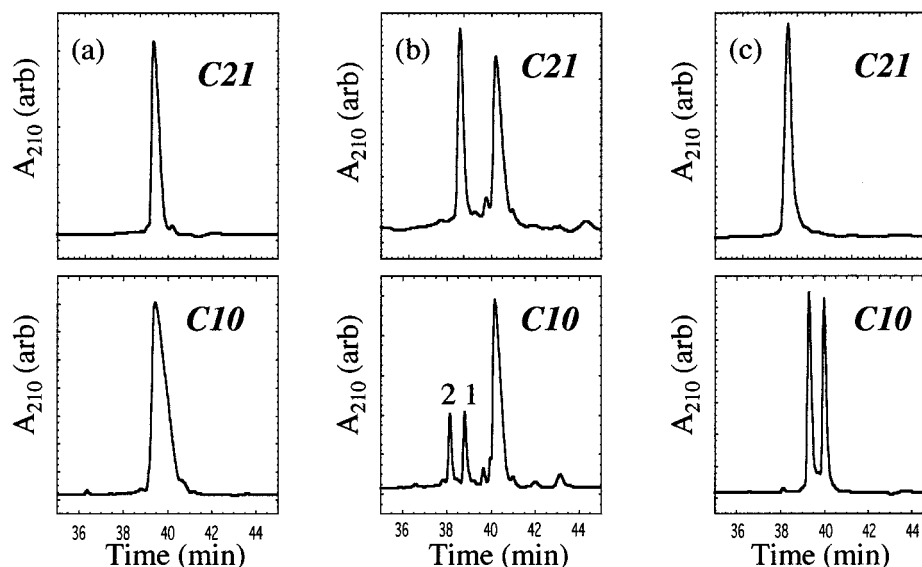
3 ([M + 4H<sup>+</sup>]<sup>4+</sup>), 782.8 ([M + 5H<sup>+</sup>]<sup>5+</sup>), 652.4 ([M + 6H<sup>+</sup>]<sup>6+</sup>), 559.4 ([M + 7H<sup>+</sup>]<sup>7+</sup>).

**Peptide Labeling with [Ru(NH<sub>3</sub>)<sub>5</sub>Cl](TFA).** The Ru(bpy)<sub>2</sub>(phen)-labeled peptides [(Δ-*l*)(Ru-phen)-C10]-H21(30-mer) and the diastereomeric mixture H10[(Δ-*l* + Λ-*l*)(Ru(phen)-C21)](30-mer) were metalated at their histidine side chains by treatment with aquopentammineruthenium(II) as described previously for the metalation of related coiled-coil peptides (3, 5). However, in the present study, the reaction mixtures were treated with 0.1 N HTFA for 2–3 h prior to purification by reversed-phase HPLC. ESI-MS *m/z* (ion): found for [(Δ-*l*)(Ru-phen)-C10][Ru(NH<sub>3</sub>)<sub>5</sub>H21](30-mer) 1366.5 ([M + 3H<sup>+</sup>]<sup>3+</sup>), 1024.4 ([M + 4H<sup>+</sup>]<sup>4+</sup>), 819.9 ([M + 5H<sup>+</sup>]<sup>5+</sup>); for [Ru(NH<sub>3</sub>)<sub>5</sub>-H10][(Δ-*l* + Λ-*l*)(Ru(phen)-C21)](30-mer) 1366.1 ([M + 3H<sup>+</sup>]<sup>3+</sup>), 1024.1 ([M + 4H<sup>+</sup>]<sup>4+</sup>), 819.8 ([M + 5H<sup>+</sup>]<sup>5+</sup>).

## RESULTS AND DISCUSSION

**Synthesis of the C10H21(30-mer) and H10C21(30-mer) Apopeptides.** Solid-phase techniques were used to prepare the 30-residue polypeptides, C10H21(30-mer): Ac-K-(IEALEGK)(ICALEGK)(IEALEHK)(IEALEGK)-G-amide, and H10C21(30-mer): Ac-K-(IEALEGK)(HIALEGK)-(IEALECK)(IEALEGK)-G-amide. These sequences are based on a seven residue heptad repeat, (*abcdefg*)<sub>*n*</sub>, in which positions *a* and *d* are occupied by hydrophobic amino acids, positions *b*, *c*, and *f* are occupied by hydrophilic residues, and positions *e* and *g* are occupied by oppositely charged residues. Such sequences have been designed by Hodges and co-workers (17) to spontaneously form two-stranded α-helical coiled-coils, which can be described as being an intertwining of 2 α-helices to form a left-handed supercoil. The amino acid sequences of C10H21(30-mer) and H10C21(30-mer) differ from one another only in their placement of the cysteine and histidine residues along the peptide chain (Figure 1). Specifically, cysteine residues were located at either position 10 or 21 of the sequence to allow coupling to a ruthenium tris(bipyridyl) complex. Histidine residues were also placed at either positions 21 or 10 of the sequence to afford the coordination of a pentammineruthenium(II) center along the same peptide chain.

**Synthesis of H10[(Ru(phen)-C21)] (30-mer).** The cysteine side chain of H10C21(30-mer) was alkylated by treatment with [Ru(bpy)<sub>2</sub>(phen-CIA)](PF<sub>6</sub>)<sub>2</sub>, where phen-CIA = 5-chloroacetamido-1,10-phenanthroline and the cysteine residue occupies the most solvent-exposed position (*f*) of the third heptad of the peptide sequence. In this procedure, the ruthenium starting material was



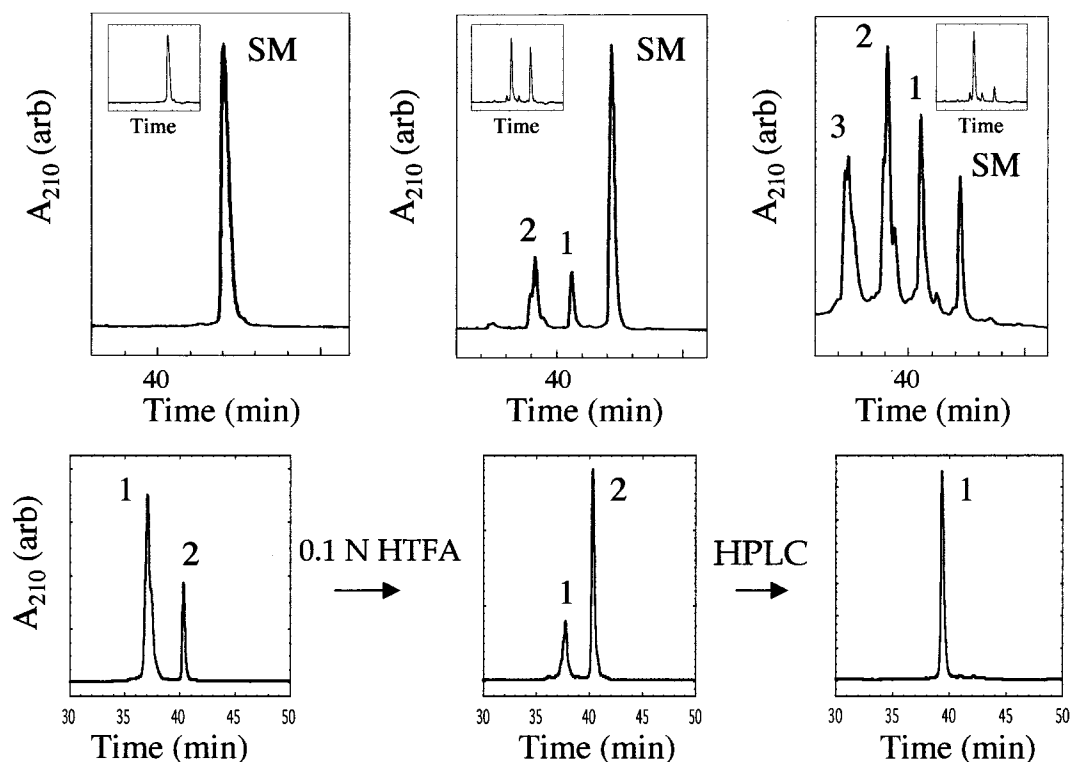
**Figure 2.** HPLC profiles monitoring the reaction between the H10C21(30-mer) peptide and  $\text{Ru}(\text{bpy})_2(\text{phen-ACl})(\text{PF}_6)_2$  (top) and between the C10H21(30-mer) peptide and  $\text{Ru}(\text{bpy})_2(\text{phen-ACl})(\text{PF}_6)_2$  (bottom): (a) apo-peptide before reaction, (b) intermediate stage of the reaction, (c) purified final product. Note that for C10H21(30-mer) two product peaks (1 and 2) are seen in the HPLC profile.

prepared by a carbodiimide coupling reaction involving chloroacetic acid and  $[\text{Ru}(\text{bpy})_2(\text{phen-NH}_2)]$ . The alkylation reaction was monitored by reversed-phase HPLC (Figure 2 (top)), which showed that the peak corresponding to the apo-peptide diminished in intensity as a new product peak appeared at a somewhat shorter retention time. Electrospray ionization mass spectrometry showed that the product had a charge-to-mass ratio consistent with that expected for H10 $[(\text{Ru}(\text{bpy})_2(\text{phen-C21})](30\text{-mer})$ . The UV-vis spectrum of the product is very similar to that of  $[\text{Ru}(\text{bpy})_2(\text{phen-CIA})]^{2+}$ , having maxima at 286 and 450 nm, but has a significantly larger absorbance at 210–220 nm due to the presence of the peptide backbone. Additional HPLC experiments showed that metalation of the H10C21(30-mer) occurs in competition with hydrolysis of the  $[\text{Ru}(\text{bpy})_2(\text{phen-CIA})]^{2+}$  starting material, as evidenced by the appearance of a new HPLC peak at shorter retention times than that of the ruthenium starting material.

The alkylation of sulfhydryl groups with 5-iodoacetamido-1,10-phenanthroline (phen-IA) has previously been used as a convenient method for attaching metal-binding sites onto the surfaces of proteins, where subsequent treatment of the modified protein with transition metal reagents has led to the creation of specific DNA cleaving agents (13, 14). In related work, luminescent probes (12) were attached to the cysteine-containing proteins human serum albumin and human immunoglobulin G, by first reacting  $[\text{Ru}(\text{bpy})_2\text{Cl}_2]$  with phen-IA and then treating the protein with the resulting metal complex. However, in our hands this preparation of the ruthenium reactant gave a mixture of two products as observed by reversed-phase HPLC. The ESI-MS of these products indicated that they were  $[\text{Ru}(\text{bpy})_2(\text{phen-IA})]^{2+}$  and  $[\text{Ru}(\text{bpy})_2(\text{phen-CIA})]^{2+}$ , of which the latter species was likely due to a halogen-exchange involving propitious chloride in solution. HPLC experiments also showed that when the mixture of  $[\text{Ru}(\text{bpy})_2(\text{phen-IA})]^{2+}$  and  $[\text{Ru}(\text{bpy})_2(\text{phen-CIA})]^{2+}$  reactants was treated with H10C21(30-mer), the iodo species hydrolyzed very rapidly and the metalation of the peptide appeared to proceed only from the  $[\text{Ru}(\text{bpy})_2(\text{phen-CIA})]^{2+}$  precursor. Indeed, when authentic samples of  $[\text{Ru}(\text{bpy})_2(\text{phen-IA})]^{2+}$  and  $[\text{Ru}(\text{bpy})_2(\text{phen-CIA})]^{2+}$  were reacted separately with the peptide, it was

found that metalation occurred only from the chloro starting material and not the iodo species. This behavior is in contrast to that reported for the metalation of human serum albumin and human immunoglobulin G in which the  $[\text{Ru}(\text{bpy})_2(\text{phen-CIA})]^{2+}$  species was observed to be nonreactive (12).

**Synthesis of  $[\text{Ru}(\text{phen-C10})\text{H21}(30\text{-mer})$ .** The cysteine side chain of C10H21(30-mer) was alkylated by treatment with  $[\text{Ru}(\text{bpy})_2(\text{phen-CIA})]^{2+}$  as described above. It is noted that the sequence of this peptide places the cysteine residue at position (b) of the second heptad repeat. In contrast to the behavior previously described for the metalation of H10C21(30-mer), it was observed that the alkylation of C10H21(30-mer) produced two distinct product peaks (Figure 2 (bottom)). The UV-vis spectra of both species were identical to that of H10 $[(\text{Ru}(\text{phen-C21})](30\text{-mer})$ , and ESI-MS showed that they both had the charge-to-mass ratio expected for the desired metalloprotein. The circular dichroism spectrum (not shown) of the faster eluting product consists of negative bands appearing at 208 and 222 nm with  $[\theta]_{222}/[\theta]_{208} = 1.04$  which indicates that the peptide exists as an  $\alpha$ -helical coiled-coil (17). These features are also observed in the CD spectrum of the H10C21(30-mer) apo-peptide (data not shown). However, the spectrum of the first ruthenated product displays an additional set of features that consists of a strong negative peak at 270 nm, a strong positive peak at 290 nm, a broad negative peak at ca. 420 nm, and a weak positive peak at 470 nm. By analogy to the CD properties of related systems, this latter set of transitions are assigned to the  $\Delta$  configuration of the octahedral ruthenium polypyridyl complex (20, 21). Thus, the faster eluting product is identified as being the  $\Delta$ -*I* diastereomer which results from coupling the chiral metal complex to the *I*-polypeptide. The CD spectrum of the slower eluting product is nearly identical to the one described above except that it displays transitions arising from the metal complex corresponding to the  $\Delta$  configuration. Thus, reaction of C10H21(30-mer) with  $[\text{Ru}(\text{bpy})_2(\text{phen-CIA})]^{2+}$  produces the two diastereomers ( $\Delta$ -*I* and  $\Delta$ -*I*) of the  $[(\text{Ru}(\text{phen-C10})\text{H21}(30\text{-mer})$  metalloprotein, which are resolvable by reversed-phase HPLC. It is noted, however, that the two diastereomers of the related H10 $[(\text{Ru}(\text{phen-C21})](30\text{-mer})$  metalloprotein



**Figure 3.** (Top) HPLC profiles showing the progress of the reaction between the H10[(Ru(bpy)<sub>2</sub>(phen)-C21](30-mer) peptide and RuA<sub>5</sub>(H<sub>2</sub>O)<sup>2+</sup> after (a) 0, (b) 1, and (c) 6 h. At longer reaction times, three product peaks are observed (1–3) along with that of the unreacted starting material (SM). The insets show the progress of the corresponding metalation reaction using the related peptide H21(30-mer) (5), which undergoes conversion to a single metalated product. (Bottom) HPLC profiles showing that the treatment of product 2 with 0.1 N HTFA results in its conversion to product 1, which can be separated by HPLC.

tide could not be separated under identical HPLC conditions. Thus, the specific placement of the ruthenium polypyridyl complex within the peptide sequence (i.e., a “b” position on C10H21(30-mer) vs a “f” position on H10C21(30-mer)) sufficiently alters the physical properties of the metalloprotein to yield significantly different HPLC properties of the two sets of metalloproteins.

**Synthesis of [Ru(NH<sub>3</sub>)<sub>5</sub>-H10][( $\Delta$ -I +  $\Lambda$ -I)(Ru(phen)-C21)](30-mer).** The histidine side chains of the H10[(Ru(phen)-C21)](30-mer) metalloprotein were coordinated to a ruthenium pentammine center by treating a diastereomeric mixture ( $\Delta$ -I +  $\Lambda$ -I) of the metalloprotein with aquopentammineruthenium(II) made in situ by reducing chloropentammineruthenium(III) chloride with zinc amalgam. Similar procedures have been used to metalate the histidine residues of a variety of native heme proteins (11) and synthetic polypeptides (3, 5, 7). As monitored by reversed-phase HPLC (Figure 3), the reaction of [Ru(NH<sub>3</sub>)<sub>5</sub>(H<sub>2</sub>O)]<sup>2+</sup> with H10[(Ru(phen)-C21)](30-mer), whose two diastereomers appear as a single HPLC peak, results in the appearance of at least three new product peaks, labeled 1–3. This behavior is in contrast to that previously observed for metalation of the related H21(30-mer) polypeptide (5) where only a single product peak appeared in the HPLC profile (insets, Figure 3).

The differential pulse polarogram of peak 1 (not shown) consists of a single reduction wave at  $-0.18$  V vs Ag/Ag<sup>+</sup> that appears at a similar potential to those of [Ru(NH<sub>3</sub>)<sub>5</sub>-H21](30-mer) (5), and Ru(NH<sub>3</sub>)<sub>5</sub> bound to His33 of cytochrome *c* (11). This peak is therefore assigned to the desired product, [Ru(NH<sub>3</sub>)<sub>5</sub>-H10][Ru(phen)-C21](30-mer). ESI-MS data support this assignment and it is noted that changes in the spectrometer voltage can yield mass spectra that are either consistent with the formulation, [Ru(NH<sub>3</sub>)<sub>5</sub>-H10][Ru(phen)-C21](30-mer), or with the com-

plete loss of the ammonia ligands, as was previously seen in the case of ruthenium ammine labeled cytochrome *c* (22).

The polarograms from peaks 2 and 3 each show two reduction waves that appear at  $-0.18$  and  $-0.32$  V vs Ag/Ag<sup>+</sup>, to suggest the presence of two types of peptide-bound ruthenium pentammine species in these samples. Based on model studies (23), the more anodic peak is assigned to the existence of pentamminecarboxylatoruthenium species bound to one or more of the glutamic acid residues in the peptide. However, the ESI-MS of 2 and 3 are identical to that of 1 and it is possible that the carboxylato bound ruthenium complexes dissociate under the MS conditions employed. Thus, to test for the existence of acid-bound ruthenium complexes, products 2 and 3 were isolated by HPLC and dissolved in 0.1 N HTFA for several hours. HPLC analysis (Figure 3) of the resulting solutions showed that this treatment produced the clean conversion of products 2 and 3 into product 1 which suggests the dissociation of carboxylato bound species at low pH.

**Synthesis of [(Ru(phen)-C10)][( $\Lambda$ -I)Ru(NH<sub>3</sub>)<sub>5</sub>-H21](30-mer).** The [(Ru(phen)-C10)]H21(30-mer) was reacted with aquopentammine ruthenium (II) as described above. However, since the HPLC profile of the starting metalloprotein was complicated by the resolution of its two diastereomers, only the  $\Lambda$ -I diastereomer was used in the reaction. The HPLC results again show the presence of multiple product peaks which can be converted to a single peak by treatment with dilute acid.

#### SUMMARY

A stepwise procedure for the preparation of site-specific, binuclear metalloproteins has been described. The peptide modification proceeds first through the

alkylation of a cysteine side-chain with  $[\text{Ru}(\text{bpy})_2(\text{phen-CIA})]^{2+}$ , followed by the coordination of a ruthenium pentammine complex to a histidine residue located elsewhere along the sequence. Circular dichroism spectroscopy shows that both the apo and metalated peptides adopt the conformation of an  $\alpha$ -helical coiled-coil in aqueous solution. When the ruthenium polypyridyl complex is attached to C10H21(30-mer), the  $\Delta$ -I and  $\Lambda$ -I diastereomers of the resulting metalloprotein are readily separated from each another by reversed-phase HPLC. However, when the reaction occurs with H10C21(30-mer), the two peptide diastereomers cannot be chromatographically resolved. These results demonstrate that the metalation of different positions of the coiled-coil sequence significantly affects the way the resulting peptide interacts with solid supports. An examination of the wheel diagram depicted in Figure 1 shows that the cysteine residue of C10H21(30-mer) is placed at a "b" position of the heptad which is located somewhat close to the peptide-peptide interface. It is speculated that the peptide-bound ruthenium polypyridyl complex may experience a restricted conformational environment that may facilitate its diastereomeric resolution by reversed-phase HPLC. In contrast, the cysteine residue in H10C21(30-mer) is located at the most highly solvent-exposed position "f" of the heptad repeat, and presumably experiences a significant degree of conformational freedom. These results indicate that the subtle interplay between peptide conformation/sequence and metal complex geometry may be used to control some of the physical characteristics of metalloproteins.

#### ACKNOWLEDGMENT

We thank Mr. Jędrzej Romanowicz for obtaining the ESI-MS data. This work was supported by the National Institutes of Health through Grant Nos. GM 61171 and GM60258 and the Petroleum Research Fund through Grant No. 34901-AC. A.F. acknowledges predoctoral support from the Harold and Helen McMaster Foundation.

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BC015544K