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Communication

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Post-Protein-Binding Reactivity and Modifications of the *fac*-$[Re(CO)_3]^+$ Core

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Supporting Information

ABSTRACT: The reactivity of the $[Re(CO)_3(H_2O)_2]^+$ complex coordinated to the His15 residue of HEW lysozyme is described. In the fully metalated protein (**Lys-1**), the Re ion retains its reactivity only toward selected ligands, while others induce a ligand-mediated demetalation of the enzyme. It is further shown that some of the complexes that may be "engineered" *on* the lysozyme do not react with the free protein even if present in solution in excess. The formation of stable metal adducts starting from **Lys-1** was confirmed by X-ray crystallography.

Chemical hybridization of proteins with non-native metal fragments constitutes an active field of research. Some groups have brilliantly directed their efforts toward the development of artificial enzymes,[1−12] while others have exploited the exogenous metal complexes in the study of long-range electron-transfer reactions or photoinduced relaxation processes.[13−16] In the case of metal ions that find applications in medicinal chemistry, the interaction of polypeptides with metal drugs is mainly investigated in order to elucidate the fate of the drug within the bloodstream or in cellular compartments.[17]

Several studies have now made available structural information of the interactions of, mainly, Pt- and Ru-based anticancer agents with cellular proteins.[18−21] In contrast, the reactivity of the protein-bound complexes has received far less attention. It is of interest, in our opinion, not only to determine the location, the structural modifications, the strength of binding, and the reversibility of protein−metal complex interactions but also to understand how the nature of the chiral protein environment might influence the chemistry of the metal complex.

This latter question has been explored only marginally. However, possible post-protein-binding reactions of, e.g., metallodrugs, might play an important role in determining the biochemical basis of the systemic toxicity of the drugs or play a role in their therapeutic effects. Elucidating the chemistry of protein-bound metal species might also offer an elegant alternative to the selenomethionine-dependent multiwavelength anomalous diffraction method for solving the so-called "phase problem".[22]

Herein we show (see Figure 1) that the chemistry of the lysozyme-bound *fac*-$[Re(CO)_3]^+$ core (**Lys-1**) is affected by the nature of the chiral protein environment when compared to the solvated metal ion [i.e., *fac*-$[Re(CO)_3(OH_2)_3]^+$ (**1**)]. The Re ion on the fully metalated enzyme **Lys-1** retains its reactivity toward selected mono- and bidentate ligands, while molecules bearing primary amines induced demetalation of **Lys-1**. The X-ray structure of the lysozyme-bound $[Re(CO)_3(OH_2)_2]^+$ complex (metal-ion occupancy modeled to 60%) was recently communicated by Ziegler and co-workers.[24,25] We found that complete metalation of the enzyme could be attained by allowing **Lys** to react with an excess of **1** (>20 equiv) for 7 days at room temperature. Mass spectrometry [MS; see the Supporting Information (SI)] clearly showed formation of the corresponding adduct (**Lys-1**) in quantitative yield.

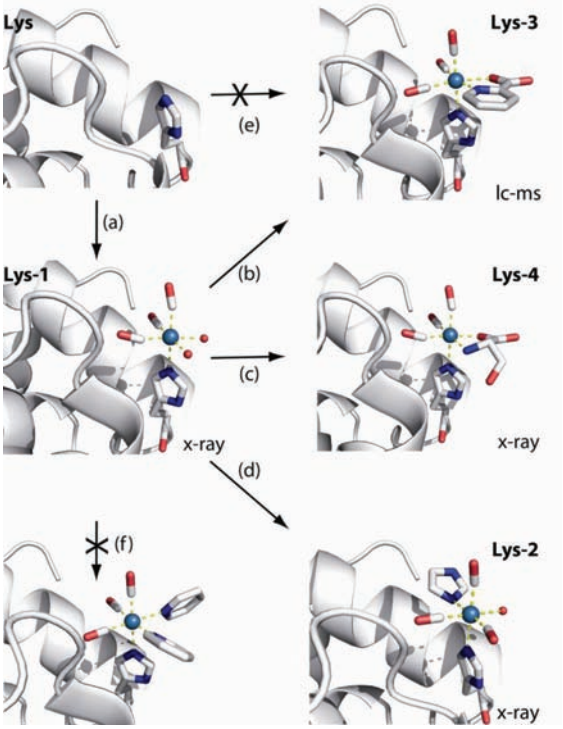

Figure 1. Reaction scheme leading to the species described in this contribution. (a) Complete metalation of HEW lysozyme (**Lys**) with complex **1**. (b−d) Derivatization of **Lys-1** with pyridine-2-carboxylic acid (pa), L-serine, and imidazole, yielding **Lys-3**, **Lys-4**, and **Lys-2**, respectively. (e) Unsuccessful synthesis of **Lys-3** via the reaction of **Lys** with the *fac*-$[Re(CO)_3(pa)(OH_2)]$ complex. (f) Reaction of **Lys-1** with pyridine showing no evidence of ligand exchange at the metal core.

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Chromatographic purification of **Lys-1** allowed isolation of the protein in ca. 80% yield with no apparent loss of the metal ion (typically on a 50 mg scale; see the SI). Circular dichroism studies and enzymatic assays on the rate of lysis of *Micrococcus lysodeikticus* showed that chromatographic purification did not affect the enzymatic activity or folding of **Lys-1** (see the SI).[26] The metalated protein thus obtained could also be crystallized, but we found no significant differences with the reported structure with the exception that Re occupancy could be modeled to a value >80%.

The 500-MHz COSY spectra of nonexchanged **Lys** and **Lys-1** are compared in Figure 2. Coordination of **1** to His15

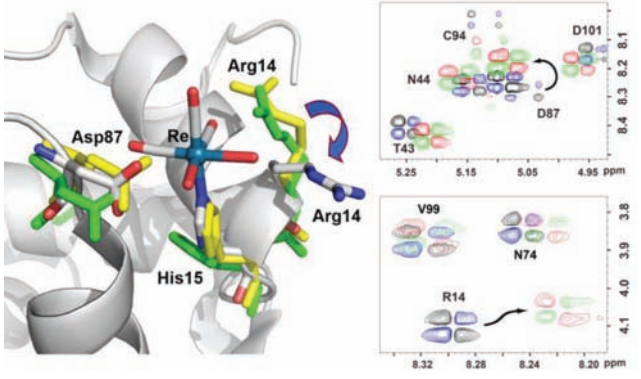

Figure 2. Left: comparison of the X-ray structure of **Lys-1** (gray, PDB entry 3KAM), a high-resolution X-ray structure of **Lys** (yellow, PDB entry 2VB1), and the NMR structure of **Lys** (green, PDB entry 1E8L). Right: selected fingerprint regions of the phase-sensitive 500-MHz COSY spectra of nonexchanged **Lys** (blue-black) and **Lys-1** (red-green). Residue assignments are as previously reported.[23]

induced a downfield shift of the resonances of the residue, which was lost under the presaturated water signal (see the SI). The most significant resonance shift was noticed for Arg14 (labeled R14 in Figure 2), an observation that may be rationalized by comparing the X-ray structure of **Lys-1** with the NMR solution structure of the free enzyme (Figure 2). The resonance changes of Arg14 are consistent with the rearrangement of the residue required to lift the steric hindrance at the binding site. Overall, the COSY cross-peak signals of **Lys-1** retained the same pattern as the ones observed for the free lysozyme (see the SI).[23]

The reactivity of **Lys-1** was studied in water by exposing the metalloprotein to different mono- and bidentate ligands. Preliminary studies of these interactions were always followed by liquid chromatography (LC)−MS. Under our experimental conditions, MS analysis of the **Lys**- and **Lys-1**-derived species always showed a distinct pattern of signals (typically four) corresponding to the 8+ to 11+ ions of the enzymes (see the SI). Changes in the position of the signal patterns offered an immediate indication of the types of interactions that resulted from the reactions of **Lys-1** (see the SI).

Reaction of **Lys-1** with imidazole (im, 10 equiv, 12 h, RT) gave the corresponding *fac*-$[Re(CO)_3(His15)(im)(OH_2)]^+$ adduct (**Lys-2**) in good yield. The coordination of a second imidazole was never observed. The X-ray structure of **Lys-2** is shown in Figure 3 (vide infra). Under similar conditions, no reaction was observed when **Lys-1** was dissolved in an aqueous solution of pyridine (py). On the other hand, reactions of the solvated metal ion **1** with im gave a mixture of products (i.e.,

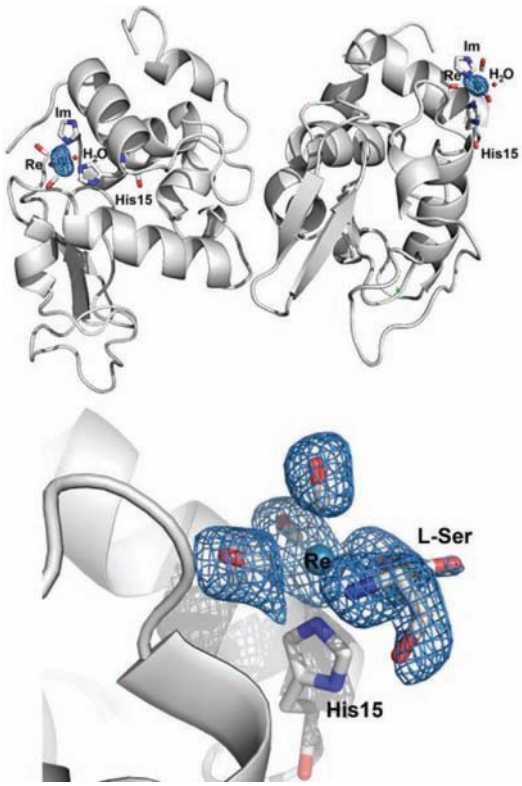

Figure 3. Top: overall structure of **Lys-2** in the asymmetric unit (anomalous electron density contoured at 4.0σ). Bottom: detailed view of the metal-binding site of **Lys-4** (difference electron density map contoured at 2.7σ; see the SI). The pictures were prepared using the *PyMOL* software.[30]

mono-, bis-, and tris-substituted complexes), and those with py gave the well-defined *fac*-$[Re(CO)_3(py)_2(OH_2)]^+$ complex.[27]

The fundamental reasons underlying these differences were not clear at this point. We speculated that hydrogen-bonding interactions between the incoming ligands and the amino acid residues in close proximity to the Re binding site may play an important role in determining the reactivity of the metal ion. To test this hypothesis, **Lys-1** was reacted with pyridine-2-carboxylic acid (pa) and 2-methylaminopyridine (pn) under the conditions described above. Reaction of **Lys-1** with pa gave the corresponding *fac*-$[Re(CO)_3(His15)(pa)]$ adduct (**Lys-3**; see the SI), but when exposed to an aqueous solution of pn, **Lys-1** was readily demetalated, resulting in a mixture of free **Lys** and the *fac*-$[Re(CO)_3(pn)(OH_2)]^+$ complex. This type of ligand-mediated demetalation of **Lys-1** was further observed in the reactions of the metalloprotein with other bidentate ligands bearing primary amines (e.g., ethylenediamine). Crystallization of samples of **Lys-3**, obtained after chromatographic purification, was attempted under a wide variety of conditions, but no diffracting crystals could be obtained.

In order to gain insight into the possible interactions of **Lys-1** with more biologically relevant molecules, the metalloprotein was also reacted with amino acids. We focused our attention on L-serine (L-ser). Under conditions similar to those described for the synthesis of the im conjugate, **Lys-1** reacted with L-ser to give the corresponding *fac*-$[Re(CO)_3(His15)(L\text{-ser})]$ adduct (**Lys-4**) in ca. 80% yield. Partial loss of the metal fragment was observed during the reaction, but no further demetalation was detected after chromatographic purification.

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Crystals of **Lys-2** and **Lys-4** suitable for X-ray diffraction analysis were obtained by the hanging-drop method (see the SI). Figure 3 shows the overall structure of **Lys-2** and a detailed view of the metal-binding site of **Lys-4**. A similar detailed view of the binding site of **Lys-2** is given in the SI. It should be mentioned that, in the case of **Lys-2**, the difference map contoured at the metal-binding site does not entirely show the imidazole ring. However, MS analysis of the single crystal employed in the X-ray diffraction analysis unequivocally points to the presence of imidazole in the Re coordination sphere (see the SI).

Lys-4 crystallized in a typical tetragonal lysozyme cell (space group $P4_32_12$, PDB accession code 3QNG). Two molecules of **Lys-2** were found in the asymmetric unit of the orthorhombic cell (space group $P2_12_12_1$, PDB accession code 3QE8). The structures were solved by molecular replacement using a **Lys** model (PDB entry 1IEE), and the restraints for the Re complexes were taken from the corresponding small-molecule structures.[28,29] In both cases, the Re complexes were refined with 80% occupancy. The crystals of **Lys-2** and **Lys-4** were diffracting until 1.55 and 1.49 Å, respectively (see the SI).

It is interesting to point out that, in the solid-state structure of **Lys-4**, a single *fac*-[Re(CO)$_3$(His15)(L-ser)] diastereomer is observed. In the structure, the OH group of L-ser is hydrogen-bonded to the threonine residue 89 (see the SI). It is possible that one of the two diastereomeric forms of **Lys-4** was selectively crystallized, or alternatively the hydrogen-bonding interaction is responsible for the binding specificity of L-ser. We tend to favor this second hypothesis because diastereomerically pure samples of *fac*-[Re(CO)$_3$(L-ser)(X)] (where X = im-type ligand) are known to rapidly epimerize at the Re center in aqueous solutions.[29]

In summary, a study of the post-protein-binding reactivity of the lysozyme-bound **Lys-1** core was presented. The study corroborated the assumption that the nature of the chiral protein environment might affect the chemistry of a metal complex on a protein surface. Our results led us to hypothesize that hydrogen-bonding interactions between the incoming ligands and the amino acid residues in close proximity to the metal-binding site may play an important role in determining the reactivity and specificity of binding of the metal ion.

■ ASSOCIATED CONTENT

Supporting Information

Experimental section, Tables S1 and S2, and Figures S1−S8. This material is available free of charge via the Internet at http://pubs.acs.org.

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