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Specific derivitization of lysozyme in aqueous solution with $Re(CO)_3(H_2O)_2^+$

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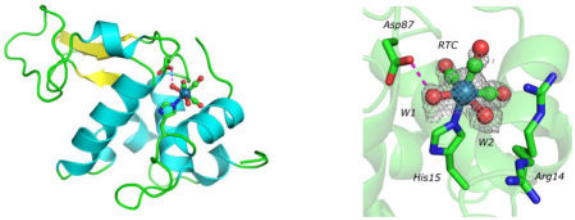
Abstract

The reaction of $Re(CO)_3(H_2O)_3^+$ with hen egg lysozyme in aqueous solution results in a single covalent adduct; single crystal X-ray diffraction shows that the rhenium tricarbonyl cation binds to His15 in two significantly populated rotamer conformations.

Graphical Contents Entry



The reaction of $Re(CO)_3(H_2O)_3^+$ with hen egg lysozyme in aqueous solution results in a single covalent adduct; single crystal X-ray diffraction shows that the rhenium tricarbonyl cation binds to His15 in two significantly populated rotamer conformations.

The aqueous chemistry of the organometallic cation $Re(CO)_3(H_2O)_3^+$ has received much attention over the past decade,[1,2] due to its relevance to the design of $^{186/188}$Re radiopharmaceuticals[3,4] as well as its ability to act as a surrogate for the chemistry of $^{99m}Tc(CO)_3^+$.[5–7] In many cases, these investigations have focused on reactions between biomolecules and $Re(CO)_3(H_2O)_3^+$.[4] Although much work has been carried out investigating interactions between $Re(CO)_3$-based compounds and nucleic acids,[8–12] amino acids and oligopeptides,[13–16] little work has been carried out on reactions between rhenium prodrug or drug model complexes and proteins. Such interactions can be crucial to the biological processing of Tc/Re based imaging agents, since proteins, rather than nucleotides or single amino acids, would be encountered in plasma. Alternatively, protein-Tc/Re adducts could be novel targets for use as imaging or therapeutic agents. In order to probe this

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chemistry, we began to examine the interactions between $Re(CO)_3(H_2O)_3^+$ and the readily crystallizable protein lysozyme.

We reacted several stoichiometries of $Re(CO)_3(H_2O)_3^+$ with hen egg white lysozyme (MP Biomedicals, 3X crystalized) in pure H_2O at 4° C overnight and analyzed these solutions by MALDI mass spectrometry.† Upon exposure to increasing concentrations of $Re(CO)_3(H_2O)_3^+$, a peak with a M/z of 14579 appears, which corresponds to addition of a $Re(CO)_3^+$ unit (270 M/z) to the mass of lysozyme (14309 M/z). At a 3:1 stoichiometric ratio of Re:protein, the 14309 M/z:14579 M/z ratio is about 60:40. Optimal protein crystals were obtained by preincubating lysozyme with a stoichiometry of 3:1 $[Re(CO)_3(H_2O)_3]Br$ to protein overnight at 4 °C. Crystals were grown from this solution by the hanging drop method in 0.05 M MES buffer, pH 5.5, 0.8 M NaCl, 50 mg/mL protein. Large tetragonal crystals formed after 48–72 hours. It is important to note that adduct formation was carried out in solution prior to crystal growth; in other cases, metal ligation is achieved only by soaking crystals in reagent solutions.

X-ray diffraction data to 1.6 Å resolution were collected at 1.54 Å (Oxford Diffraction Gemini R) and was integrated and scaled using CrysalisPro (99.6% complete, R_{int} = 0.091, $I/\sigma(I)$ = 47.2, redundancy = 22.4). The structure was solved by molecular replacement (Phaser) using PDB 6LYZ and subjected to several cycles of restrained refinement using Refmac5 and rebuilding in Coot.[17–20] The final structure (PDB 3KAM, R = 0.181, R_{free} = 0.215, rms bonds = 0.015 Å, rms angles = 1.6°) contained clearly identifiable electron density for $Re(CO)_3(H_2O)_2^+$ attached to Nε2 of His15. The occupancy of the $Re(CO)_3(H_2O)_2^+$ fragment in the model is less than 1.0 and was modeled at 0.60, consistent with the MALDI-MS data which indicates partial derivatization of the protein.

The refined Re-O bond distance for water W2 is somewhat long (2.6 Å compared to the expected 2.2 Å as observed for W1), suggesting that there are at least two significantly populated rotamers of the $Re(CO)_3(H_2O)_2^+$ adduct, related by a 90° rotation about the Re-N bond. In the final model, however, only the principal rotamer was modeled. Partial occupancy of a CO ligand at the W2 position results in the Re-O bond having an artificially long bond distance. The observation that the electron density of the $Re(CO)_3(H_2O)_2^+$ fragment is well-ordered, rather than averaged because of free rotation about the Re-N axis, is possibly due to the stabilizing hydrogen bond contributed by Asp87. In the principal rotamer, Asp87 interacts with W1; in the minor rotamer, Asp87 interacts with W2. The Re-N distance of 2.2 Å compares well to related small molecule structures.[21] While it is not possible to definitively rule out partial occupancy of chloride ion at position W2 of the rhenium tricarbonyl complex to explain the anomalously long Re-water bond length at this position, we believe the simplest interpretation of the data is the existence of two rotamers with water coordinated at positions W1 and W2.

The presence of $Re(CO)_3^+$ adducts in these single crystals was confirmed by IR spectroscopy. Figure 2 shows the spectra of native hen egg lysozyme before exposure to

†Electronic supplementary information (ESI) available: MALDI mass spectra of native hen egg lysozyme reacted in aqueous solution with various stiochiometries of $Re(H_2O)_3(CO)_3^+$ and full spectra of the data shown in Figure 2 see DOI: 10.1039/b000000x

$Re(CO)_3(H_2O)_3^+$ and of lysozyme crystals grown from the 3:1 reaction described above. In the 1800–2200 cm^{-1} region, unmodified lysozyme is transparent, but the $Re(CO)_3^+$ modified crystals show ν(CO) stretching bands corresponding to e and a_1 modes (1890, 2001 and 2013 cm^{-1}) resulting from the pseudo-C_{3v} symmetry of the facial $Re(CO)_3^+$ unit. These bands do not correspond to those of the $Re(CO)_3(H_2O)_3$ species, which shows an a_1 band at 2036 cm^{-1}.[22] The appearance of two a_1 bands is consistent with the presence of multiple rotamers of the $Re(CO)_3^+$ adduct as described above.

To the best of our knowledge, the above described work is the first example of a crystallographically characterized $Re(CO)_3(H_2O)_2^+$-protein conjugate. There is a significant body of work on the ligation of non-natural metal ions to the periphery of lysozyme, such as the binding of cisplatin to the His15 site.[23] More recently, organometallic complexes have been attached to this and other proteins, including Fischer-type metallocarbenes,[24] $Mn(CO)_3(H_2O)_2^+$,[25] half-sandwich Ru(II) species[26] and most notably Re(diimine)$(CO)_3^+$ adducts which were used for long distance electron transfer studies and also have been crystallographically characterized.[27] In addition to the medicinal relevance of our work, the peripheral modification with $Re(CO)_3^+$ could be used to further the fundamental characterization of proteins. With regard to X-ray structural elucidation, $Re(CO)_3(H_2O)_3^+$ could be used as a heavy atom reagent to assist crystal phasing, since it meets all the essential requirements of phasing agents.[28] The cation contains a heavy atom, is water soluble, doesn't hydrolyze, is relatively non-toxic, has selectivity since it can readily form a covalent bond with a surface histidine but is not expected to react with other common groups such as amines and thioethers, and binds to the protein in a way that does not alter the crystal lattice of the protein. Alternatively, $Re(CO)_3(H_2O)_3^+$ could be used to generate the aforementioned Re(diimine)$(CO)_3^+$ type adducts. The ligation of Re(diimine)$(CO)_3$X or Re(diimine)$(CO)_3(H_2O)^+$ to the periphery of proteins can require more than three weeks of reaction time at 37 °C.[27] In the current work, we observed that $Re(CO)_3(H_2O)_3^+$ reacts with the protein at 4 °C in as little as twelve hours. Subsequent reaction of the conjugate with a diimine would result in the desired electron transfer conjugate. We are continuing our work in the area of protein-$Re(CO)_3(H_2O)_2^+$ interactions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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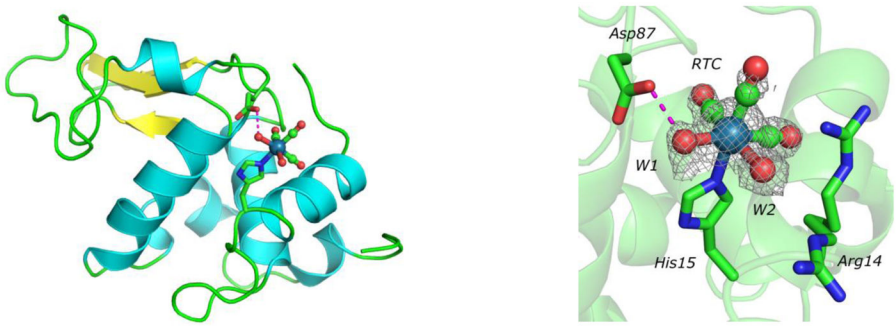

Fig 1.

Left: Ribbon diagram of hen egg white lysozyme with $Re(CO)_3(H_2O)_2^+$ bound to its unique site at Nε2 of His15. Hydrogen bond between Asp87 and water W1 of $Re(CO)_3(H_2O)_2^+$ is depicted as a magenta dashed line. Right: *F*o-*F*c omit map of lysozyme-$Re(H_2O)_2(CO)_3^+$ (RTC) adduct, contoured at 2.5σ. Arg14 has weak electron density and has alternate conformations, and does not appear to interact with $Re(H_2O)_2(CO)_3^+$.

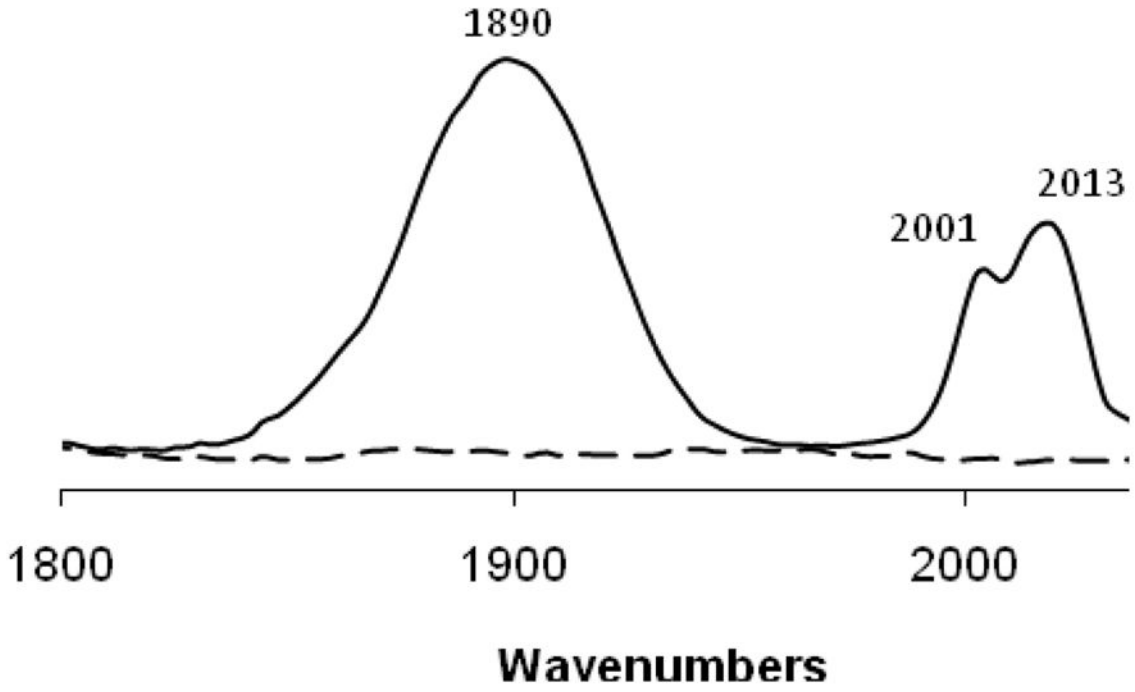

Fig. 2.

IR spectra of the ν(CO) stretching region of native hen egg white lysozyme (dashed line) and $Re(CO)_3^+$ modified lysozyme (solid line).†