Tricarbonylmanganese(I)–lysozyme complex: a structurally characterized organometallic protein†

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The reaction of the new and structurally characterized covalent $\{Mn(CO)_3(H_2O)_2\}^+$–lysozyme adduct with NiS_4 and NiN_2S_2 complexes generates binuclear Ni–Mn complexes; relevance to the reactivity of the protein-bound $\{Fe(CO)(CN)_2\}$ intermediate during maturation of [NiFe] hydrogenases is discussed.

Organometallic proteins are currently emerging as a new class of chemical compounds of interest. One reason resides in the demonstration that covalent or non-covalent incorporation of an organometallic complex into a protein is feasible and this paves the way for the preparation of novel hybrids combining properties of both organometallic and protein chemistry.[1–12] More recently it became obvious that fascinating albeit rare examples exist in nature. In particular metal-carbonyl moieties have been discovered at the active site of Fe-only,[13,14] [NiFe][15–19] and iron–sulfur cluster-free (H_2-forming methylenetetrahydromethanopterin dehydrogenase)[20–22] hydrogenases. It has been shown that during maturation of [NiFe] hydrogenases, a $\{Fe(CN)_2(CO)\}$ fragment is first assembled before coupling to a nickel ion.[23,24] Artificial organometallic derivatives of proteins as potential structural models of these maturation proteins might help in understanding how the interactions with peptidic residues stabilize and even control the reactivity of soft organometallic centers in aqueous solutions. However there is so far only one structurally characterized hybrid system, combining a protein and a metal carbonyl moiety, namely $\{Re(CO)_3phen(His\text{-}azurin)\}$,[25–27] coordinated by a protein-derived amino acid. We decided therefore to investigate the reactivity of a variety of water-soluble iron, ruthenium and manganese carbonyl complexes‡ towards lysozyme. This protein was selected on the basis of its reported ability to incorporate a $\{Ru(p\text{-cymene})\}^{2+}$ center.[12] In most cases, however, binding to lysozyme was not specific and no defined adduct could be isolated. We present here the synthesis, purification, spectroscopic and structural characterization of the $\{Mn(CO)_3\}^+$ derivative of lysozyme. Furthermore, we have found that this compound is able to transfer the $\{Mn(CO)_3\}^+$ group to a nickel complex, thus forming a structural mimic of the active site of [NiFe] hydrogenases similar to the functional nickel–ruthenium electrocatalysts previously described.[28,29]

Reaction of hen egg white lysozyme with excess $[Mn(CO)_3(OH_2)_3]^+$ (**1**), formed *in situ* by aquation of $[Mn(CO)_3(acetone)_3](CF_3SO_3)$ (**2**)[30] in 50 mmol L^{-1} acetate buffer, pH 4.4, yields a covalent $\{Mn(CO)_3\}^+$–lysozyme adduct (**3**) which can be purified by size-exclusion gel chromatography.§ The resulting light yellow solution displays new infrared ν_{CO} absorption bands at 2040 (m), 1942 (vs) and 1910 (s) cm^{-1} (Fig. 1) clearly different from those of **1** (2050 (s, A_1) and 1942 (vs, E) cm^{-1}). Compound **3** was shown by atomic absorption to contain at least 0.6 Mn atom per polypeptide chain.

In order to characterize this novel artificial organometallic protein structurally, we reacted crystalline lysozyme with complex **2** and collected X-ray data from a derivatized crystal at a synchrotron source.¶ The 1.7 Å resolution structure (Fig. 2) reveals that only one organometallic moiety, modelled as $\{Mn(CO)_3(OH_2)_2\}^+$, is bound per protein molecule with an occupancy of 0.8.‖ The organometallic moiety is bound to the Nε of the single histidine (His 15) residue of lysozyme as in the arene–ruthenium derivative previously reported.[12] The average B-factor for the complex is 29 $Å^2$, similar to the average value of His15 which is 28 $Å^2$. The Mn–N distance of 2.4 Å is in the usual range of Mn–N bond lengths in complexes. The manganese ion has octahedral coordination with three CO ligands in facial

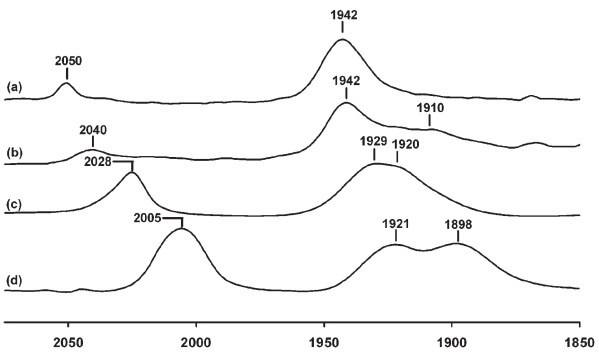

Fig. 1 FTIR spectra of (a) $[Mn(CO)_3(OH_2)_3]^+$ (**1**) in ammonium acetate buffer (50 mmol L^{-1}; pH 4.4, AgCl cell, 25 μm), (b) **3** in ammonium acetate buffer (50 mmol L^{-1}; pH 4.4, AgCl cell, 25 μm), (c) crystals of **3** (KBr pellet), (d) complex **4** (KBr pellet).

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† Electronic supplementary information (ESI) available: Detailed experimental procedures, ESI-MS and ^1H NMR spectrum of $[Ni(xbsms)Mn(CO)_3(OH_2)]^+$. See DOI: 10.1039/b703887a

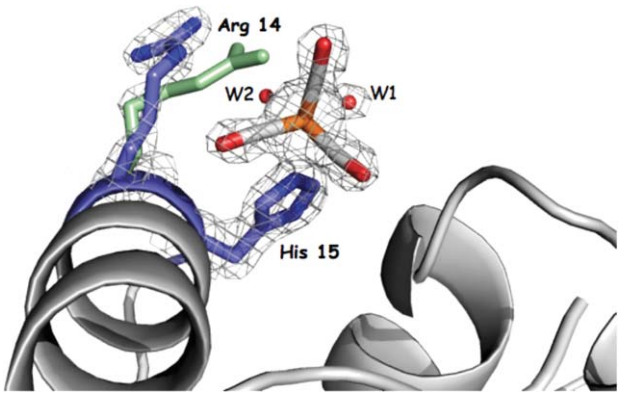
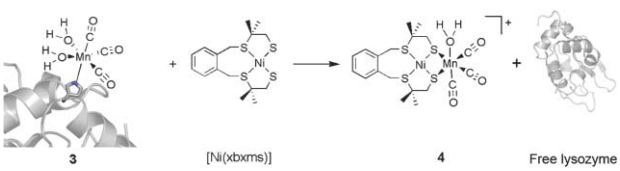


Fig. 2 X-Ray crystal structure of [(lysozyme)$Mn(CO)_3(OH_2)_2$] (**3**) – Electron density around manganese, carbonyl, His15, Arg 14 and aquo ligands. The 2|Fo| − |Fc| map is contoured at the 1σ level. W2 is disordered or not fully occupied as indicated by its lower electron density. The manganese ion is depicted in orange and the position of Arg 14 in the uncomplexed lysozyme is also shown (green). Main distances (Å) and angles (°): Mn–N: 2.4; Mn–W1: 2.3; Mn–W2: 2.3; average Mn–C: 1.9; average Mn–O: 3.0; Mn–W1: 83; N–Mn–W2: 75; N–Mn–C1: 93; N–Mn–C2: 173; N–Mn–C3: 85; C1–Mn–C2: 90; C1–Mn–C3: 91; C3–Mn–C2: 88. This figure was prepared with Pymol.[31]

arrangement and two aquo ligands (W1 and W2 in Fig. 2) *cis* to each other. W2 appears disordered or not fully occupied but is clearly seen in the 2|Fo| − |Fc| map contoured at the 0.5σ level (not shown). Comparison with native lysozyme shows that $\{Mn(CO)_3(OH_2)_2\}^+$ binding has very little effect on the general protein folding apart from residue Arg14 which moves away from the Mn site as a consequence of electrostatic repulsion between positive charges.

As compared to the infrared spectrum of **1**, which displays the A_1 + E pattern typical for *fac*-tricarbonyl coordination in octahedral complexes, the solid state infrared spectrum of the crystals of **3** (KBr pellet) with $A_1\nu_{CO}$ band at 2028 cm^{-1} and splitting of the E band (1929 cm^{-1} and 1920 cm^{-1}) indicates some loss of symmetry upon coordination to lysozyme. The infrared spectrum obtained upon dissolution in ammonium acetate buffer is identical to the one shown in Fig. 1b thus confirming the identity between the species formed in the crystal soaking experiment and during reaction in solution.

In the natural maturation system, the interaction of the chaperone HypC with the precursor pre-HycE of the large subunit of hydrogenase makes the $\{Fe(CO)(CN)_2\}$ fragment accessible for reaction with a Ni-binding protein (Hyp B) and assembly of the Ni–Fe active center.[23,24] In a similar way, the metal-carbonyl moiety in **3** is quite solvent-exposed. This led us to investigate the reactivity of **3** toward Ni complexes. Reaction of **3** with [Ni(xbsms)] (H_2xbsms = 1,2-bis(4-mercapto-3,3-dimethyl-2-thiabutyl)benzene)[32] cleanly yields **4** formulated as



Scheme 1 Synthesis of **4** through reaction of **3** with [Ni(xbsms)].

$[Ni(xbsms)Mn(CO)_3(OH_2)]^+$ (Scheme 1), which could be extracted in CH_2Cl_2 and characterized by infrared spectroscopy ($\bar{\nu}_{CO}$ = 2005 (s), 1921 (s) and 1898 (sh) cm^{-1}, Fig. 1d) and the ESI-MS technique ($[M - H_2O]^+$: m/z = 541(100%), see ESI), and free lysozyme. By comparison, reaction of **1** with [Ni(xbsms)] under the same conditions is far less selective, yielding a mixture of products, among them **4** as the major product. If one equivalent of imidazole (Im) is added as a histidine surrogate to the aqueous solution of **1** prior to the addition of [Ni(xbsms)], the reaction product is $[Ni(xbsms)Mn(CO)_3(Im)]^+$ as shown by mass spectroscopy (m/z = 609) and IR spectroscopy ($\bar{\nu}_{CO}$ = 2014, 1924 and 1902 cm^{-1}). The different outcomes (in one case the N-based ligand is retained in the reaction product and in the other it is not) illustrate the impact of the protein on the reaction.

Reactions of **3** with smaller nickel thiolate complexes such as $[Ni(emi)]^{2-}$ and $[Ni(ema)]^{2-}$ (H_2emi = *N*,*N*′-ethylenebis(2-mercaptoisobutyramide), H_2ema = *N*,*N*′-ethylenebis(2-mercaptoacetamide))[33] also yield binuclear Ni–Mn complexes. In this case also purification on desalting columns (Sephadex G25 or NAP10) allowed the separation of a colorless lysozyme solution from a reddish-brown fraction containing the binuclear complex (ν_{CO} absorptions at 2010 and 1920 cm^{-1}).

The protein–organometallic hybrid **3** is a unique complex for the following reasons. (i) It is the first structurally characterized tricarbonylmanganese(I)–aquo complex with a monodentate organic ligand and its stability is probably due to some steric protection by the polypeptide chain. (ii) The manganese-carbonyl fragment can react with nickel-thiolate complexes to form novel Mn–Ni complexes which will be further studied with regard to their electrocatalytic properties for H_2 production. To the best of our knowledge, this is the first report of the synthesis of binuclear complexes with the $\{Ni(\mu\text{-}SR)_2Mn(CO)_3\}$ core. (iii) As one of the very rare structurally characterized protein-bound metal-carbonyl species, it might help in understanding the structure and the reactivity of the protein-bound $\{Fe(CO)(CN)_2\}$ intermediate during maturation of [NiFe] hydrogenase.

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Notes and references

‡ Tricarbonylmanganese(I) chemistry shows similarities with tricarbonyliron(I) chemistry, namely the formation of thiolate bridged dimetallic hexacarbonyl clusters.[34,35]

§ All the reaction, purification and characterization steps are carried out in the dark because of the light sensitivity of $\{Mn(CO)_3\}^+$-containing species in aqueous solution.

¶ Lysozyme crystals were grown at room temperature within one day using the hanging drop method. The reservoir (0.5 mL) was a 0.05 mol L^{-1} acetate buffer pH 4.4 solution containing 0.9 mol L^{-1} NaCl. Each drop was a mixture of 2 µL of the reservoir solution and 2 µL of a solution of lysozyme (50 mg) in pure water (1 mL).

Lysozyme crystals were soaked in mother liquor (50 mM sodium acetate pH 4.4, 1.1 M NaCl) with about 1 µL of the oily **2** in the dark. After one week, the deep-yellow crystals were cryo-protected by soaking in artificial mother liquor with 25% (v/v) glycerol and flash-cooled using liquid nitrogen.

‖ Data were collected at beamline BM-30 of the European Synchrotron Radiation Facility (ESRF) in Grenoble, France. Short exposure times (1 s/image) were used because of the light-sensitivity of **3**. Data reduction

was carried out using XDS.[36] The structure was solved with REFMAC[37] by the molecular replacement method using the atomic coordinates of a lysozyme X-ray structure (PDB code 193L). Crystallographic refinement was conducted using REFMAC and the three-dimensional model was examined and modified using the graphics program O.[38] The coordinates have been deposited in the Protein Data Bank (PDB) under the accession code 2Q0M.

Data statistics: space group, $P4_32_12$; unit cell: $a = b = 79.542$ Å, $c = 36.392$ Å; $\alpha = \beta = \gamma = 90^\circ$; resolution 1.7 Å, observed reflections (unique), 44446 (11852); I/σ (last bin), 20.61 (5.57); completeness (last bin)%, 88.60 (84.10); R_{sym} (last bin), 3.50 (22.70); Refinement: R/R_{free}%, 21.10/26.10; rmsd bonds 0.006 Å/angles 1.087°; average isotropic B, 24.51 Å^2.

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