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Half-sandwich arene ruthenium(II)–enzyme complex

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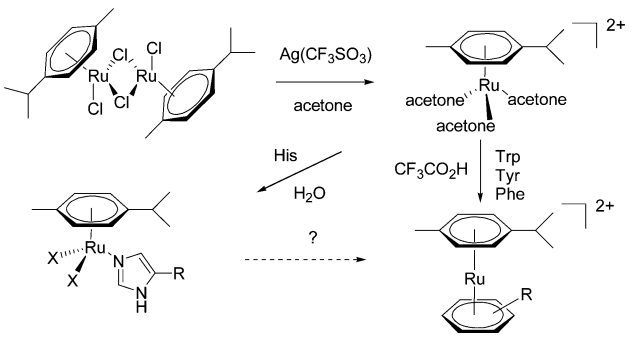
The 1.6 Å X-ray crystal structure of [(η⁶-*p*-cymene)Ru(lysozyme)Cl_2], the first of a half-sandwich complex of a protein, shows selective ruthenation of Nε of the imidazole ring of His15.

There is increasing interest in exploration of biological organometallic chemistry.[1–3] Organometallic adducts of proteins in particular may possess novel and useful properties. For example, protein derivatization can provide a useful heavy-atom labelling for solving the 'phase problem'.[4] Recent success in labelling the enzyme lysozyme with organometallic complexes for this purpose has been achieved by Jaouen *et al*. by selective covalent modification with organometallic pyrylium ions bearing arene chromium tricarbonyl[5] or ruthenocenyl groups,[6] the latter yielding derivatives modified at various lysine residues. Certain organometallic complexes containing cyclopentadiene (Cp) or arene ligands have been shown to exhibit anticancer activity,[7–9] and it is important to determine the nature of their binding sites on proteins in order to understand their mechanisms of action. Further interest in the design of novel arene Ru(II) centres arises from their potential for catalytic activity.[10]

Here we report studies of reactions of η⁶-*p*-cymene Ru(II) complexes with hen egg white lysozyme. Lysozyme is a single-chain protein of 129 amino acid residues (MW 14 kDa) and contains 4 cystine disulfide bonds. In our initial studies, we reacted lysozyme with excess (*ca*. 20 mol equiv) [(η⁶-*p*-cymene)-$Ru(acetone)_3]^{2+}$ in trifluoroacetic acid (see Scheme 1). Under such conditions, in which competing nucleophilic sites are protonated, this Ru(II) arene complex is known to form η⁶ π-complexes with model aromatic amino acid derivatives, such as *N*-acetyl-tryptophan.[11] After dialysis to remove unbound and weakly bound Ru, a product was obtained with 9.4 mol Ru per mol lysozyme (analysis by ICP-MS). This suggested that π-complexes may indeed be formed by several of the 13 aromatic side-chains in the protein (Trp, Phe and Tyr), some of which *e.g*. Trp62 and Trp63 appear to be readily accessible from the protein surface. However, we were unable to crystallize this adduct for structure determination.

In a second approach, we soaked tetragonal lysozyme crystals† by the addition of tiny individual crystals of [(η⁶-*p*-cymene)-$RuCl_2(H_2O)$]‡ to a 5 μl drop in which lysozyme crystals had grown. Transferral of these Ru(II) crystals was continued over a period of 2 days until it was observed that the lysozyme crystals had turned dark yellow in colour. The ruthenated lysozyme crystals were then removed in a cryo-loop, frozen in liquid N_2 with type-B immersion oil as a cryoprotectant, and X-ray diffraction data were collected.§ Details of the data collection and structure refinement are given below.¶



Scheme 1 Reactions of half-sandwich Ru(II) arene complexes.

In the X-ray structure, Ru(II) is bound to lysozyme as a half-sandwich complex, selectively to Nε of the imidazole ring of the only histidine residue in the protein, His15, with a Ru–N distance of 2.21 Å. The Ru–C distances for the coordinated arene, *p*-cymene, are 2.21–2.39 Å. These distances can be compared to those for the reported complex[12] [(η⁶-*p*-cymene)Ru(L-His-methyl ester)Cl]Cl for which Ru–N is 2.063 Å and Ru–C 2.159–2.171 Å. Electron density for the two additional ligands bound to Ru(II) was modelled as chlorine (although partial occupancy by water cannot be ruled out). In this model the Ru–Cl distances are 2.34 and 2.43 Å and the chloride ligands are H-bonded to water (as shown in Fig. 1c). His15 is situated on the surface of the protein (Fig. 1), and ruthenation has little effect on the rest of the enzyme structure. The root-mean-square-difference fit of all backbone atoms is 0.22 Å compared with the native structure (193L). The main chain atoms for residues Arg14 and His15 move by up to 0.4 Å from their positions in the native structure and the side-chains for these two residues adopt notably different conformations to accommodate the Ru complex. There are no other significant structural perturbations. The average *B*-factor for the complex is 21 $Å^2$ and is similar to the average values of the surrounding side chains (Asp87 (21 $Å^2$), His15 (20 $Å^2$) and Arg14 (30 $Å^2$)). The (multiple) conformations adopted by Arg14 provide the Ru arene complex with a rather hydrophobic binding pocket.

There appear to be no other structures of half-sandwich Ru(II) arene protein complexes in the protein database (pdb), although the structures of several adducts of other Ru(II) complexes have been determined. The derivatisation of histidine residues with $\{Ru(NH_3)_5\}^{2+}$, for example, provides photoexcitable centres for studies of electron transfer pathways.[13] It is possible that binding to histidine is the kinetic product from reaction of lysozyme with {(*p*-cymene)Ru}$^{2+}$, and that slow conversion to a more thermodynamically stable adduct π-bonded to an aromatic side-chain could occur. Fish *et al*. have shown that reactions of Cp and $Cp^*Ru(CH_3CN)_3^+$ with methylpyridine and quinoline tend to give N-coordinated complexes as kinetic products which undergo intramolecular isomerization to give π-coordinated pyridine complexes.[14] Similarly Grotjahn *et al*.[15] have found that the 27-residue protein secretin reacts with a tethered $\{CpRu(II)\}^+$ complex in water to give a η⁶-arene complex involving a phenylalanine residue on the protein, possibly *via* migration of Ru from a monodentate binding site. In the present case the nearest aromatic side-chain is that of Phe3, *ca*. 8 Å away, and there is no evidence for such a π-complex being formed.

The selective binding of {(η⁶-*p*-cymene))Ru(II)Cl_2} to the imidazole of His15 of lysozyme observed here, and the nature of the environment of the Ru centre, suggest that such sites can provide a basis for the design of novel catalytic centres. Currently such catalytic centres in arene Ru(II) complexes are known to require careful control of the asymmetry of Ru(II), ligand lability

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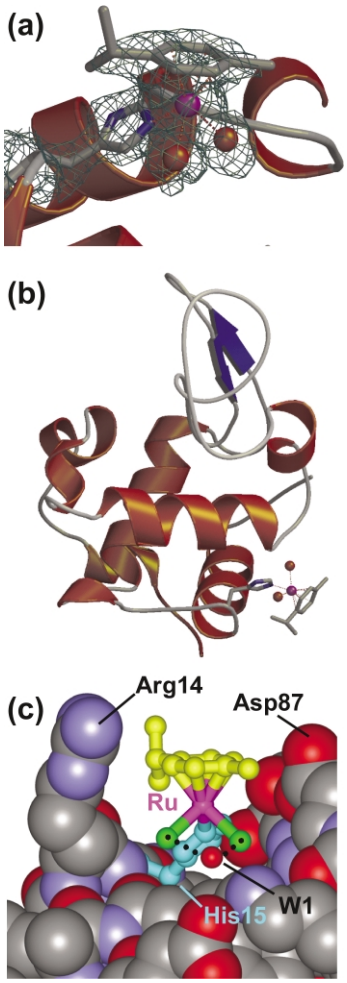

Fig. 1 X-Ray crystal structure of [(η⁶-*p*-cymene)Ru(lysozyme)Cl_2]. (a) Electron density around ruthenium, the π-bonded arene (*p*-cymene), His15 and two chloride ligands. The $2|F_o| - |F_c|$ map is contoured at 1s. (b) Secondary structure of the protein showing the position of the half-sandwich Ru(II) complex in the structure. (c) Space-filling model of the protein binding pocket with ball and stick model of the coordination sphere of Ru together with H-bonded water (W1) Colour codes: *p*-cymene yellow, His15 cyan, C grey, N blue, O red, Ru purple; Cl green (H-atoms omitted).

and acidity.[10,16,17] Site-directed mutagenesis and recombinant DNA technology would allow fine control of the electrostatic and hydrophobic environment of coordinated {(arene)Ru(II)}$^{2+}$ anchored by side-chains such as His15 in lysozyme. Protein derivatisation with arene complexes could also be used to enhance the hydrophobicity of a protein face (*e.g.* for chromatographic separations) and for solubilising proteins in non-aqueous media. In this context it is notable that the behaviour of proteins in low dielectric media is becoming well understood.[18]

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Notes and references

† Lysozyme crystals were grown at 277 K using the hanging drop method. The reservoir was a mixture of 0.05 M sodium acetate buffer, pH 4.5 (100 μl), saturated NaCl solution (200 μl) and water (700 μl), and the drop contained hen egg white lysozyme (50 mg ml^{-1}) in acetate buffer (2.5 μl) and reservoir solution (2.5 μl).

‡ Initially we added a solution of [(η⁶-*p*-cymene)Ru(acetone)$_3$](CF$_3$SO$_3$)$_2$ in acetate buffer to the lysozyme solution in the crystallisation well. New tiny crystals (of a Ru(II) complex) were seen to form within 5 min. Analysis by positive-ion electrospray-ionisation mass spectrometry of the bright yellow solution formed by dissolving such crystals in CH_3CN gave peaks at *m*/*z* 270.4 and 311.5 assignable to {(η⁶-*p*-cymene)RuCl}$^+$ (calc. *m*/*z* 270.0) and {(η⁶-*p*-cymene)RuCl(CH_3CN)}$^+$ (calc. *m*/*z* 311.0), respectively, suggesting that the crystals are probably [(η⁶-*p*-cymene)$RuCl_2(H_2O)$].

§ Data were collected at station 14.2 at the SRS Daresbury and processed using the programs MOSFLM and SCALA.[19] The initial structure was solved using a reported lysozyme structure (pdb code 193L), and refinement performed using the program REFMAC.[20] Manual checking and correction were performed with the program O.[21] The coordinates have been deposited in the Protein Data Bank (PDB) under the accession code 1T3P.

¶ *Data statistics:* space group, $P4_32_12$; unit cell, 80.14 Å, 80.14 Å, 37.06 Å, 90°, 90°, 90°; Resolution range (high shell), 37–1.6 (1.69–1.6) Å; molecules per asymmetric unit, 1; observed reflections (unique), 114339 (16497); I/σ (high shell), 2.3 (2.4); completeness (high shell) %, 99.9 (99.9); multiplicity (high shell), 6.9 (7.1); R_{sym} (high shell) %, 8.3 (31.5);

Refinement: R/R_{free} %, 18.2 / 21.7; RMS bonds (Å)/angles (°), 0.012/1.422; average isotropic *B*, 15.348.

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