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Ruthenium(III) readily abstracts NO from L-arginine, the physiological precursor to NO, in the presence of H_2O_2. A remarkably simple model system for NO synthases

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Reaction of [Ru(Hedta)Cl]$^-$ with H_2O_2 in the presence of arginine, produces NO, in the form of an Ru(II)–(NO$^+$) complex and citrulline which is a remarkably simple model system for the physiological NO synthase reaction.

The physiological importance of nitric oxide is now firmly established.[1] The processes in which it plays a pivotal role include regulation of cardiovascular function, signalling between nerves in the peripheral and central nervous systems, mediating host defence against bacteria and tumour cells and many others.[1] In living organisms NO is generated by oxidation of the guanidine function of L-arginine, a reaction which involves two sequential monooxygenations and proceeds *via* the stable intermediate *N*-hydroxy-L-arginine ultimately affording L-citrulline and NO. The entire process is catalysed by haem-containing NO synthases and requires NADPH and O_2. Despite the physiological importance of NO the biosynthetic pathway of this important reaction is not yet fully understood. Analogous to the well established cytochrome P450 *N*-oxidation chemistry, the first monoxygenation step is thought to be mediated by a transient, high valent iron–oxo porphyrin complex, which hydroxylates L-arginine and forms enzyme bound *N*-hydroxyarginine.[1,2] The proposed second monooxygenation step involves nucleophilic attack by a peroxo ligand attached to ferric haem on the guanidine carbon giving a tetrahedral intermediate from which NO and citrulline are eliminated. This step however, which has no known precedent in biological chemistry, is much less understood than the first,[1] and there are many unanswered questions relating to the fine mechanistic detail of the reaction. The ability of ruthenium(III) complexes to form oxoruthenium(V) species with the potential to hydroxylate substrates,[3] such as arginine and the affinity of the resulting reduced ruthenium(III) species for NO,[4,5] prompted us to explore systems such as these as models for NO synthase, Fig. 1. One such system based on the well characterised complex K[Ru(Hedta)Cl] and utilising H_2O_2 in place of NADPH and O_2 is described in this communication.

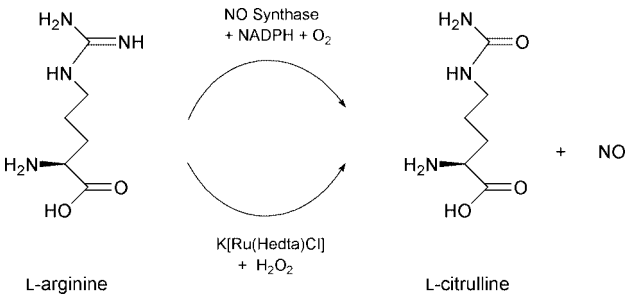

Fig. 1

Reaction of K[Ru(Hedta)Cl][3,6] with a four-fold excess of L-arginine in the presence of hydrogen peroxide in aqueous solution, gave in a highly exothermic reaction, a brown solution which, following purification on a Sephadex LH 20 column and removal of solvent, afforded a brown product† having an intense ν(NO) IR band at 1888 cm^{-1} indicative of a linear, diamagnetic Ru^{2+}–NO$^+$ containing complex.[4,7]‡ The product of denitrosylation of arginine was shown by TLC and by mass spectrometry to be citrulline, identical to the physiological denitrosylation product, eqn. (1).§

$$\text{Ru(III)} + \text{arginine} + H_2O_2 \rightarrow \text{Ru(II)–NO}^+ + \text{citrulline} + H_2O \quad (1)$$

A likely reaction mechanism involves initial hydroxylation of arginine to *N*-hydroxyarginine by the green Ru^V=O complex¶ which is formed by oxidation of the ruthenium(III) complex with hydrogen peroxide. This is remarkably similar to the first monooxygenation step of the NO synthase reaction but involves oxoruthenium(V) instead of a high valent oxoiron species and H_2O_2 as a surrogate active oxygen donor instead of NADPH and O_2. The oxidation of *N*-hydroxyarginine to citrulline and NO in our system may occur by a 'peroxide shunt' mechanism similar to that previously reported by Marletta and coworkers where H_2O_2 supported oxidation of *N*-hydroxyarginine, in the presence of NO synthase, yields the same products.[8] In our system the Ru(III) complex fulfils the role of NO synthase as in the first monooxygenation step. Alternatively in the presence of Ru(III), NO abstraction from *N*-hydroxyarginine may occur by a mechanism similar to that previously proposed for NO abstraction from hydroxamic acids,[5] involving hydroxylamine (a known source of NO ligands)[9] as an intermediate, and analogous to an earlier mechanism proposed by DeMaster *et al.* for the second NO synthase-mediated monooxygenation step.[10]

In summary we have conclusively shown that in the presence of hydrogen peroxide and [Ru(Hedta)Cl]$^-$ arginine releases NO and is converted into citrulline, a reaction identical to that occurring physiologically. The precise structures of the ruthenium complexes involved in the reaction sequence as well as the reaction mechanism are currently being investigated.

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Notes and references

† Reaction of an aqueous solution of K[Ru(Hedta)Cl]·2H_2O (400 mg, 0.80 mmol) with an aqueous solution of L-arginine (557 mg, 3.2 mmol) in the presence of an excess of H_2O_2 (40 mL, 30%) gave a highly exothermic reaction with an initial colour change from deep red to green and then to brown. The brown solution was reduced in volume to approximately 10 mL, passed through a Sephadex LH20 column and the brown fraction taken to dryness (*ca.* 71% yield without attempted optimisation).

‡ Microanalytical and other data are consistent with the formula HArg-[Ru(Hedta)(NO)Cl]·H_2O in which the cation is protonated arginine, present in excess in the reaction medium. Found: C, 29.42; H, 4.64; N, 15.43. HArg[Ru(Hedta)(NO)Cl]·H_2O ($C_{16}H_{30}N_7O_{12}ClRu$) requires C, 29.61; H, 4.66; N, 15.11%.

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§ The reaction mixture, on completion of reaction, when analysed by TLC (silica plates and an ethanol–water–ammonia (8 : 1 : 1) solvent mixture), confirmed the presence of citrulline having an R_f value identical to that of an authentic sample. The mass spectrum of an aqueous citrulline solution has a peak at *m*/*z* 176.2, also present in the spectrum of the reaction mixture (K[Ru(Hedta)Cl] + L-arginine + H_2O_2) but not in the reaction mixture in which H_2O_2 is not present (all solutions buffered at pH 8.4).

¶ The green solution gave a visible spectrum with an intense band having λ_{max} at 390 nm characteristic of a $\pi^*(O) \rightarrow t_{2g}$ Ru^V charge transfer band very similar to that in the spectrum of K[Ru(v)=O(edta)].[3]

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