Electrochemical Studies on Purified Rhus Vernicifera Laccase

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The simplest members of the multi-copper oxidase family are laccases, capable of catalysing the four-electron reduction of oxygen to water. Recently, we have simplified the purification method for Rv laccase using hydrophobic interaction chromatography, to yield a single band on SDS-PAGE.

During the past decade, alkanethiol monolayers (SAM's) have been extensively used to modify gold surfaces for electrostatic and covalent protein immobilisation. However, quality reports of direct protein electrochemistry have been limited; cytochrome c and azurin being notable exceptions. We have successfully immobilised laccase purified by the above method onto a gold electrode using a 3-mercapto propionic acid SAM followed by activation with EDC/NHS. Using this technique, amines on the laccase form a covalent amide bond with carbonyl groups previously immobilised on the electrode surface. This has enabled us to undertake a comprehensive study of the electrochemical properties of laccase. Our study has highlighted a concerted 4-electron transfer at slow scan rates (v) under anaerobic conditions, involving all four copper atoms within the protein, as assessed by peak current ratios (ipa/ipc). Addition of micromolar concentrations of the known laccase inhibitors N3- and F- resulted in a significant reduction in peak current ratio, indicative of binding to the type 2 (T2) or type 3 (T3) copper atoms. This study suggests that azide binds to two of the copper atoms at the triangular active site, probably one T3 copper atom and the T2 copper atom. In the presence of a potential difference, the complexed copper atoms are lost from the protein, resulting in a second redox wave at lower potential. Under unbuffered conditions, catalytic currents were observed in the presence of the biological substrate oxygen, emphasising the retention of *in vivo* function throughout the immobilisation process.

Metal Carbonyls – A New Class of Pharmaceuticals?

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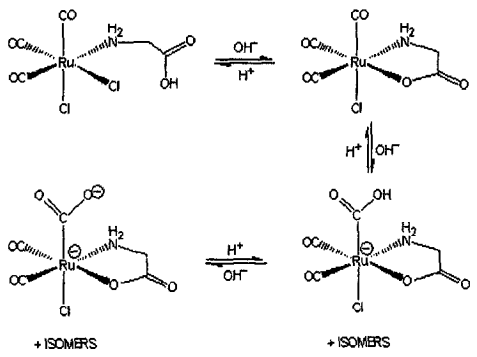
Carbon monoxide, a notoriously dangerous gas, is produced naturally by the human body and has recently been shown to possess important biological functions. In a similar fashion to nitric oxide, CO has been identified as a signalling molecule in mammals. Furthermore, CO gas has been shown to be biologically beneficial as it elicits vasodilatation, suppresses organ graft rejection and reduces the damaging effects caused by ischemia. Metal carbonyl compounds, a long established area of chemistry, could be used as CO carriers and potentially be developed as pharmaceuticals for the therapeutic delivery of CO in humans.



This poster largely focuses on the chemistry of compounds belonging to the class of $[Ru(CO)_3Cl(amino\ acidate)]$, organometallic complexes of low toxicity. The amino acids used are enantiomerically pure; however, the fac- arrangement of the ligands results in metal chirality. Preliminary results show the glycinate complex to be a highly effective CO carrier functioning as a solid form of CO that can be rapidly released in biological systems. Mechanistic studies have therefore concentrated on this species.

Compounds of this type have proved to exhibit rich aqueous chemistry. Ligands are easily replaced resulting in CO loss, whilst the CO ligands are readily attacked and undergo Water-Gas Shift reactions (see above). NMR studies have identified a number of compounds that are formed, depending on pH and temperature.

Tony R. Johnson, Brian E. Mann, James E. Clark, Roberta Foresti, Colin Green and Roberto Motterlini, Angew. Chem., Int. Ed. Engl., in press.