Electrochemical Reduction of Human Methaemoglobin

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Summary The electrochemical reduction of human methaemoglobin with the formation of deoxyhaemoglobin and possible intermediates is reported.

HUMAN deoxyhaemoglobin (Hb) can be chemically oxidized to methaemoglobin (Hb^+) where each of the four iron atoms per molecule have been converted from the Fe^{II} into the Fe^{III} state. Deaerated solutions of Hb^+ yield one or more well-defined polarographic waves depending on the supporting electrolyte used. In the buffer,† pH = 6·8, two reduction waves are observed with half-wave potentials of −0·75 and −1·04 V *vs.* S.C.E. Other reduction waves are observed but they are very ill-defined. The well-defined waves are both partially kinetically controlled as indicated by the dependence of their limiting currents on mercury pressure[1] and their relative responses to the vibrating dropping mercury electrode (V.D.M.E.) and the D.M.E.[2] *S* values for the first and second waves respectively, in the buffer, are 0·00 and 0·35, where *S* is defined by the equation: $i_L = kh^S$ and i_L is the limiting current of the wave, h is the corrected mercury pressure, and k is a constant. R values for the two waves are 0·00 and 0·26 respectively, where R is the ratio of the V.D.M.E. to D.M.E. limiting currents. A V.D.M.E. frequency of 190 Hz corresponding to ms drop times was used.

In polarograms of Hb^+ in 0·1M KCl (no buffer present), the first wave is observed with a half-wave potential of −0·85 V *vs.* S.C.E.; this reduction wave is apparently kinetically controlled since $R = 0$. The second wave is absent but the other ill-defined reduction waves are observed at potentials negative of the first wave and positive of the solvent reduction wave.

The second polarographic wave, observed in the buffer, is probably due to the reduction of some amino acid fraction rather than the heme since it is present in polarograms of both Hb^+ and Hb. The fact that the second wave is not observed in the KCl solutions indicates that it probably arises from differences in the tertiary or quaternary structure of the protein in the two solutions. Possibly some amino acid fraction is more susceptible to electroreduction in the buffer.

Controlled potential electrolysis at a mercury pool electrode maintained at −1·00 V *vs.* S.C.E. (a potential on the plateau of the first wave) of a 0·1M KCl solution of Hb^+ results in a *one*-electron per mole reduction of Hb^+. Controlled-potential electrolysis, under the same conditions, at −1·30 V results in a 1·5 electron per mole Hb^+ reduction before cessation of current flow.

Controlled-potential electrolysis under the same conditions but at −1·50 V *vs.* S.C.E. results in a 4·0 electron per mole Hb^+ reduction before the current reaches a low constant level characteristic of the supporting electrolyte. This corresponds to the conversion of all four atoms per mole of Hb^+ from the Fe^{III} into the Fe^{II} state. The solution resulting from this electrolysis is dark red-brown which when agitated in the presence of air quickly changes colour to a bright clear red. The pH of the solution was 5·0 ± 0·1 throughout the electrolysis; the protein concentration was 0·1mM.

The u.v.-visible spectrum of the aerated red product is identical with that of HbO_2.[3] The starting material agreed well with the reported spectra of Hb^+.[3]

Studies are now being undertaken on the electrogeneration and electrochemistry of partially reduced haemoglobin species containing both Fe^{II} and Fe^{III} oxidation states.

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† Tris(hydroxymethyl)aminomethane (0·05M), cacodylic acid (0·05M), and perchloric acid (0·10M) adjusted to pH 6·8 with NaOH.

[1] P. Zuman, 'Organic Polarographic Analysis,' Pergamon Press, New York, 1964, pp. 22—25.
[2] R. E. Cover and J. G. Connery, *Analyt. Chem.*, 1969, **41**, 918.
[3] DMS Ultraviolet Atlas of Organic Compounds,' Plenum Press, New York, 1966, Volume II.