ELECTROCHEMICAL REDUCTION OF DIOXYGEN USING A TERMINAL OXIDASE

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1. Introduction

The reduction of dioxygen to water is a reaction central to aerobic life. Much is known about the terminal oxidases of both prokaryotes and eukaryotes. They contain at least two redox centres and are efficiently coupled to the redox proteins which preceed them in the electron-transport chain. Coincidentally with the interest in the biological reduction of dioxygen to water, much effort has been expended [1] in the search for efficient 'oxygen' electrodes. Non-enzymic reduction of dioxygen to water is [2] often slow and usually involves a complex series of steps. Elsewhere we have [3,4] drawn attention to the possible parallels between electron transport at an electrode surface and that in biological systems. We have reported [5] an attempt to couple electron transport between a surface-modified gold electrode and a mammalian cytochrome oxidase. We now describe the successful coupling of electron transport between a gold electrode, upon which 1,2-bis(4-pyridyl)ethene is adsorbed [6–9], and the soluble terminal oxidase/nitrite reductase of *Pseudomonas aeruginosa*.

2. Materials and methods

2.1. *Protein purification*

Horse heart cytochrome *c*, type III, was obtained from the Sigma Chemical Co. It was purified [10] to remove all polymeric and de-amidated forms by ion-exchange chromatography using carboxymethylcellulose resin (CM32, Whatman Biochemicals Ltd). *Pseudomonas aeruginosa* cytochrome oxidase/nitrite reductase and *P. aeruginosa* cytochrome c_{551} were purified according to [11].

2.2. *Other materials*

1,2-Bis(4-pyridyl)ethene was obtained from the Aldrich Chemical Co. and recrystallised from ethanol/water. All supporting electrolyte reagents were of Aristar grade.

2.3. *Electrochemistry*

DC cyclic voltammograms were obtained using an Oxford Electrodes potentiostat and were recorded on a Bryans XY recorder 26000 A3. The cell was of all glass construction, incorporating a conventional 3 electrode system. The gold working electrode, a 4 mm diam. disc, supplied by Oxford Electrodes Ltd, was polished before each experiment using an alumina (particle size 1 μm)/water slurry on cotton wool and then washed with distilled water. The reference electrode was a saturated calomel electrode (SCE) and the counter electrode a platinum gauze. The bulk reduction experiment was carried out in a closed perspex system of volume 7.0 cm^3 incorporating a gold grid working electrode, (the generous gift of Johnson Matthey Ltd), an SCE reference electrode connected by a Luggin capillary, and a platinum gauze counter electrode in a side-arm behind a fine glass frit. The oxygen concentration was monitored using a YSI 5331 Oxygen probe supplied by the Yellow Springs Instrument Co. The solution was stirred magnetically and the grid maintained at −0.3 V *vs* SCE to give rapid electron transfer.

3. Results and discussion

The DC cyclic voltammogram of horse heart cytochrome *c* is shown in fig.1(a). This cyclic voltammogram, showing both forward and reverse peaks, ~60 mV apart, is typical of a reversible electron-

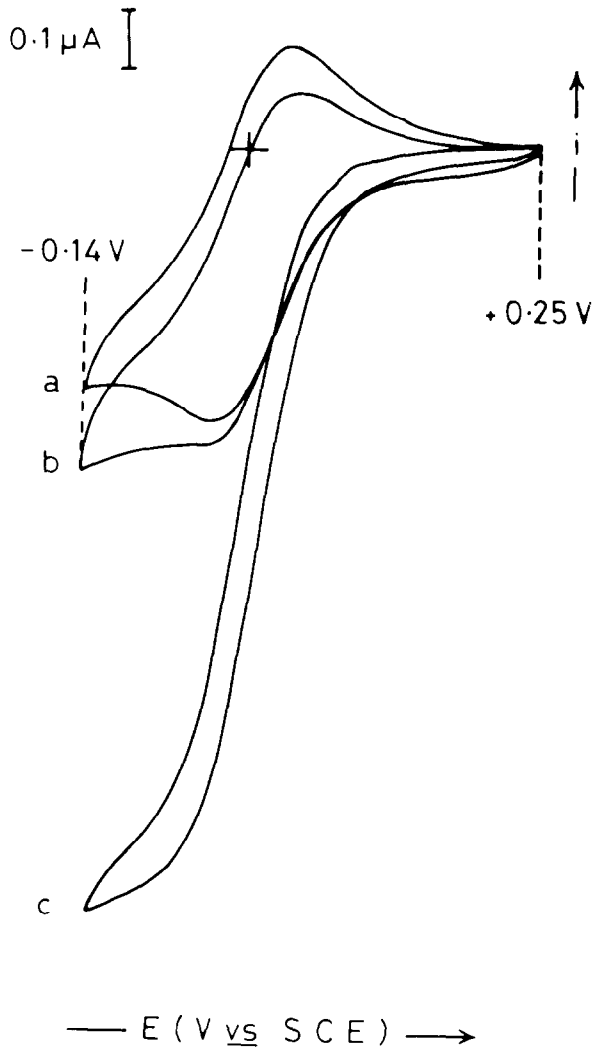

Fig.1. DC cyclic voltammogram of 0.75 cm^3 $NaClO_4$ (0.1 M), phosphate buffer (0.02 M) (pH 7), 1,2-bis(4-pyridyl)ethene (1 mM), with successive additions of: (a) horse heart iron(III) cytochrome *c* (4 mg); *P. aeruginosa* cytochrome oxidase (0.77 μM); and (c) *P. aeruginosa* cytochrome c_{551} (0.55 mg). DC potential scan rate, 1 mV/s, in the range −0.14 to +0.25 V *vs* SCE.

transfer process. The peaks do not appear in the absence of 1,2-bis(4-pyridyl)ethene or related compounds [3–9]. We have shown [3,4,6–9] that these compounds, which are themselves neither oxidised nor reduced at these potentials, are adsorbed from solution on to the electrode surface. Cytochrome *c* binds to this modified surface and electron transfer ensues. On the addition of the cytochrome oxidase

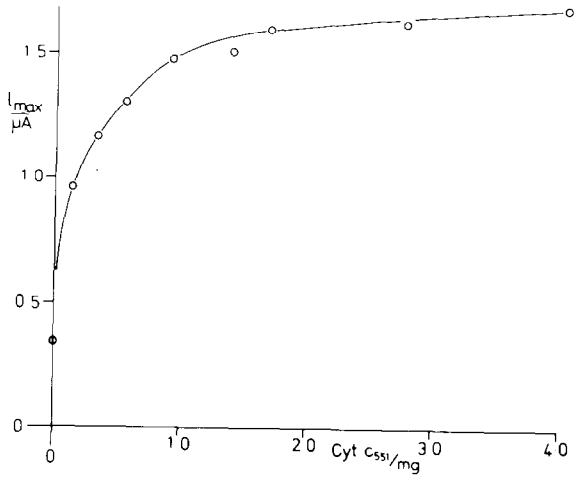

Fig.2. Variation of the maximum current at a potential of −0.14 V *vs* SCE, i_{max}, with mass of cytochrome c_{551} added to the solution (conditions as in fig.1b).

from *P. aeruginosa* under aerobic conditions a small change in the cyclic voltammogram is observed (fig.1,b). This is consistent with the reported rate [12,13] of the reaction between these two proteins. However, on the addition of cytochrome c_{551} from the same bacterium a dramatic change in the DC cyclic voltammogram is observed (fig.1,c). Under

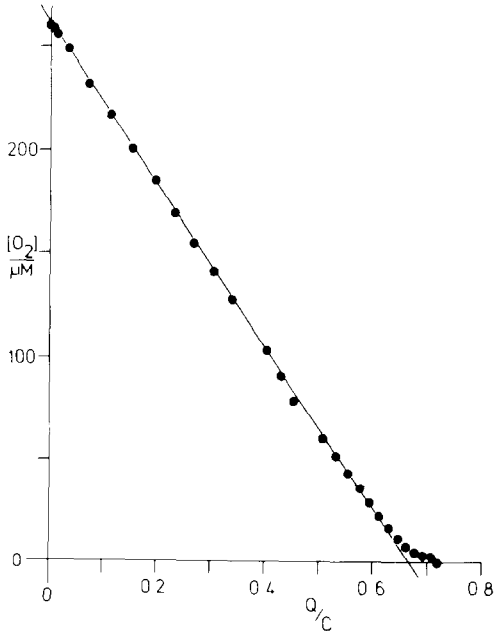

Fig.3. The dioxygen concentration ($[O_2]$) as a function of the charged passed/coulombs. Conditions are described in the text.

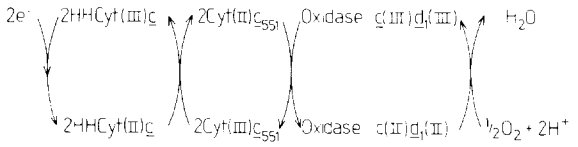

Fig.4. The proposed electron transfer sequence; HHCyt c = horse heart cytochrome c.

these conditons we have found the rate of electron transfer between the electrode and cytochrome c_{551} is slow.) However, the rates of electron transfer between horse heart cytochrome c and cytochrome c_{551} on the one hand [14,15] and the latter protein and the cytochrome oxidase on the other [16] are fast. The size of the current (fig.1,c) is dependent on the cytochrome c_{551} concentration as shown in fig.2.

Under anaerobic conditions, the original, reversible voltammogram of horse heart cytochrome c is reproduced (fig.1,a). The relationship between the amount of charge passed and the dioxygen concentration for a bulk reduction experiment is shown in fig.3. The number of coulombs passed is equivalent to 3.8 Faradays/mol dioxygen. These observations are consistent with the electron transfer sequence shown in fig.4.

A detailed analysis of the data will be published later. These results are sufficient to show that it is possible to efficiently couple electron transport at an electrode to the reduction of dioxygen to water via a terminal oxidase.

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