CHEMISTRY LETTERS, pp. 1145-1148, 1977. Published by the Chemical Society of Japan

REVERSIBLE ELECTRODE REACTION OF CYTOCHROME c

Peter YEH and Theodore KUWANA
Department of Chemistry
The Ohio State University
Columbus, OHIO 43210 USA

The heme protein, cytochrome c, was found to exhibit reversible electron transfer characteristics at an indium oxide electrode. The electrode reaction at this electrode was evaluated using cyclic voltammetry and differential pulse method.

Previous electrochemical studies[1,2] of cytochrome c have exhibited only irreversible characteristics which could be rationalized by assuming that the large peripheral protein structure hindered facile electron-transfer between the electrode and the heme iron center. Thus, most recent redox studies of cytochrome c have been conducted using mediator-titrants which couple the electron transfer from the electrode to cytochrome c[3,4].

As part of our research work[5,6] to develop so-called "tailor-made" electrodes by chemically modifying the surface for selective and/or catalytic electrode reactions, we were greatly surprised to observe a reversible appearing current-voltage (i-E) curve by cyclic voltammetry when cytochrome c was electrolyzed at a "control" indium oxide electrode. Initial consideration was given to the possibility that the i-E curve was partially due to a phenomenon associated with either surface adsorption of cytochrome c or denatured material. However, subsequent examination to be described herein has supported the contention that a diffusion controlled, reversible electrode reaction is occuring at the indium oxide electrode. To our knowledge, this is one of the first instance of observing a reversible electrode reaction for a heme protein[7].

Cyto c[8] solutions were prepared from stock by dilution with phosphate buffer (ionic strength = 0.15 M, pH = 7.0). Cyclic voltammetric (CV) and differential pulse voltammetric (DPV) measurements were made using a Princeton Applied Research Model 174A polarographic analyzer on thoroughly degassed solutions which were contained in a cell previously described[9]. The tin-doped indium oxide electrode was obtained from Pittsburgh Plate Glass Co., Pittsburgh, PA. All potentials are referenced to a silver/silver chloride (1.00 M KCl) electrode. Activity of cyto c was ascertained by titrations with cytochrome

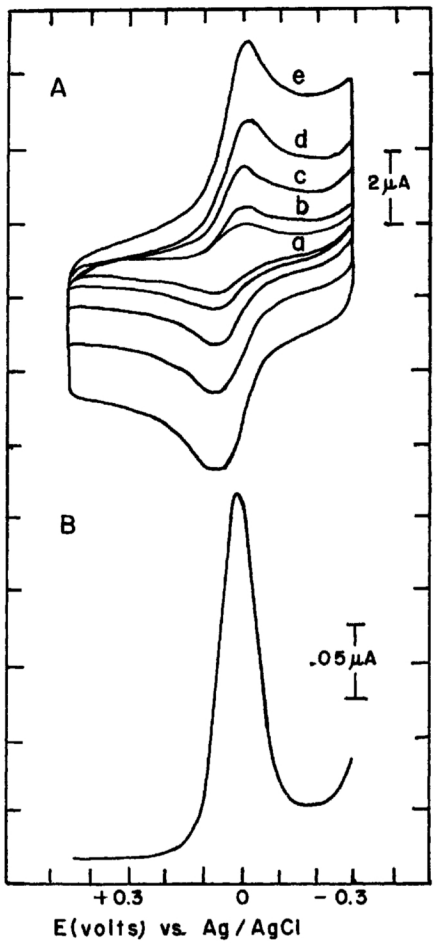

Fig. 1 A. Cyclic i-E curves of 52 μM cyto c at 10, 20, 50, 100, and 200 (a to e) mV/s. B. Differential pulse i-E curve of 20 μM cyto c at 2 mV/s scan rate, 50 mV pulse height, and 0.5 s pulse width.

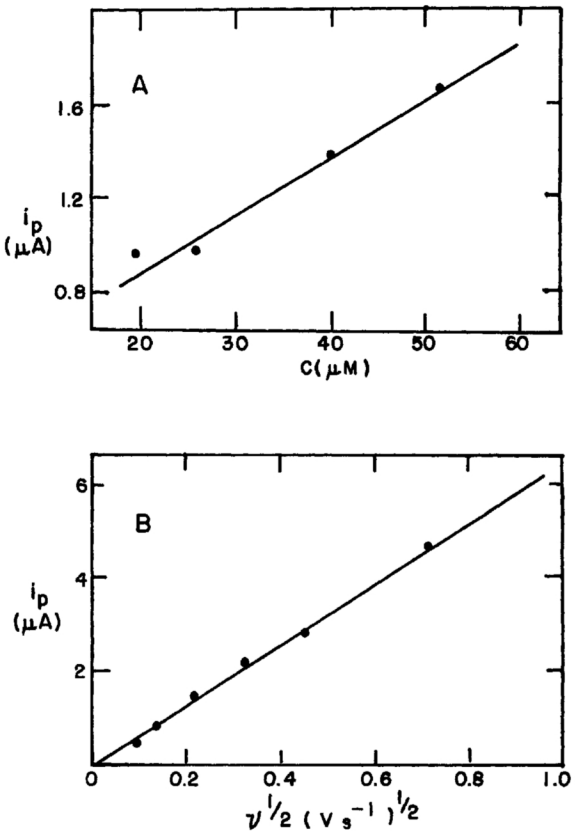

Fig. 2 A. Plot of cyclic peak currents (at 50 mV/s) as a function of cyto c concentration. B. Plot of cyclic peak currents as a function of scan rate (52 μM cyto c).

c oxidase[10].

A typical cyclic i-E curve is shown in Fig. 1A for the reduction of a 52 μM cyto c solution at an indium oxide electrode. In the potential range of +0.45 to -0.30 volt, a well-defined voltammetric wave characteristic of a diffusion controlled electrode reaction is observed. The ΔE_p separations between the cathodic (E_{pc}) and anodic (E_{pa}) peak potentials varied between 60 and 70 mV which are very close to the theoretical value[11] of 58 mV for an one electron reaction. The ΔE_p remained essentially independent (± 10 mV) of scan rate in the range of 10 to 500 mV/s. The $(E_{pa} + E_{pc})/2$ value was in excellent agreement (± 5 mV) of the $E^{o'}$ value (+ 0.257 ± 0.017 V vs NHE)[12] which has been determined by potentiometry or the indirect coulometric titration method[9] in the presence of mediators. The cathodic peak currents

(i_{pc}) were linearly proportional to cyto c concentrations at a constant scan rate (see Fig. 2A) and also to the square root of scan rates at a constant cyto c concentration (see Fig. 2B) as expected for a diffusion limited electrode reaction. The plots in both figures intersected the origin when the i_{pc} values were corrected for the background capacitive currents. The diffusion coefficient of cyto c calculated from the slope of the i_p vs cyto c concentration gave a value of 5×10^{-7} cm^2/s. This value is considerably lower than those values (11×10^{-7} cm^2/s) determined by other methods and have been reported in the literature[13, 14]. The reason for the difference is not known at present.

Because of the high sensitivity afforded by DPV, experiments using this method were performed to confirm the CV results. A well-defined, symmetric peak-shaped DPV wave was observed at ca. 0.0 volt vs Ag/AgCl (see Fig. 1B). The I_p (DPV) heights were also proportional to cyto c concentration.

Cyto c also exhibited cyclic i-E waves at the tin oxide electrode although the behavior was much less reversible. Spectroelectrochemical experiments at these metal oxide, optically transparent electrodes are being pursued to further characterize the electrochemical behavior of cyto c and other heme proteins.

We are grateful for the financial support provided by U. S. National Institutes of Health grant GM 19181. This paper was written during tenure of T. K. as a visiting professor at the Pharmaceutical Institute, Tohoku University, Sendai, Japan, under the sponsorship of the Japan Society for Promotion of Science. The kind hospitality of Professor T. Osa is greatly appreciated.

REFERENCES

1) S. R. Betso, M. H. Klapper, and L. B. Anderson, J. Am. Chem. Soc., 94 (1972) 8197.
2) L. Griggio and S. Pinamonti, Atti. Ist. Veneto Sci. Lett. Arti. Cl. Sci. Mat. Natur., 124 (1965-66) 15.
3) T. Kuwana and W. R. Heineman, Bioelectrochem. and Bioenerg., 1 (1974) 389.
4) Y. Fujihira, T. Kuwana, and C. R. Hartzell, Biochem. Biophys. Res. Commun., 61 (1974) 488.
5) A. W. C. Lin, P. Yeh, and T. Kuwana, submitted to Anal. Chem.
6) J. F. Evans and T. Kuwana, J. Electroanal. Chem., in press.
7) The reversible electrode reaction of cytochrome c_3 has been recently reported by K. Niki, et al. (U.S./Japan Seminar, May 1977, San Francisco).
8) Purified cytochrome c sample was received from C. R. Hartzell (Pennsylvania State University, University Park, PA.).

9) F. M. Hawkridge and T. Kuwana, Anal. Chem., 45 (1973) 1021.
10) J. L. Anderson, T. Kuwana, and C. R. Hartzell, Biochemistry, 15 (1976) 3847.
11) R. S. Nicholson and I. Shain, Anal. Chem., 36 (1964) 706.
12) Average of 19 literature values (see p. 1026 of reference 9).
13) H. Theorell, Biochem. Z., 285 (1936) 207.
14) A. Ehrenberg, Acta Chem. Scand., 11 (1957) 126.

(Received August 6, 1977)