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Direct Voltammetric Behavior of Rs. rubrum Cytochrome c' at a 2-Mercaptosuccinate-modified Gold Electrode

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The direct electrochemical redox behavior of cytochrome c' was first observed at a surface-modified gold electrode by 2-mercaptosuccinate. Cytochrome c' showed quasi-reversible redox responses at the electrode, and the heterogeneous rate constant for the redox reaction was estimated to be the order of 10^{-4} cm/s.

It is well-known that redox proteins do not show rapid direct voltammetric responses at normal metal electrodes, except for some examples such as cytochrome (cyt.) c_3.[1] However, various functional electrodes, on which rapid electron transfer of redox proteins can take place, have been developed during the past decade.[2] Recently, we[3,4] have found that cyt. c_2 from Rhodospirillum rubrum showed the quasi-reversible voltammetric responses at a 4-mercaptopyridine-modified gold electrode, but questions for the interactions between the promoter and the protein remain unanswered. Cyt. c' from Rs. rubrum is similar in the isoelectric point and the heme binding sequence pattern to those of cyt. c_2, but differs in heme iron ligation and spin state, and overall backbone fold.[5,6] The interactions between promoters and redox proteins may, therefore, be elucidated from the similarities and differences between the voltammetric results obtained with cyt's c' and c_2, if a suitable promoter can be found for the direct electrochemistry of cyt. c'. The present paper deals with the preliminary voltammetric behavior of cyt. c' at surface-modified gold electrodes, prior to the elucidation of the promoter-protein interactions.

Cyt. c' was prepared from light-grown cells of Rs. rubrum according to the method described previously.[7] Cyt. c' thus prepared was dissolved in 0.1 M (= mol/dm^3) Tris-HCl buffer (pH 8.0), and the concentration was determined by measurements of absorbance at 390 nm. Cyclic voltammetry was carried out at various concentrations of cyt. c' and potential sweep rates

as described.[3] Surface modification of a gold disk was carried out by dipping the freshly polished electrode into 1 mM aqueous solution (or saturated solution if indissolved) of modifiers for 10 min.

Twelve compounds, 4-mercaptopyridine, 4-mercaptophenol, 4-aminopyridine, 4-mercaptomethoxybenzene, (4-pyridylthio)acetic acid, L-methionine, 2-mercaptoethanic acid, 3-mercaptopropionic acid, 3,3'-thiodipropionic acid, 2,4-dinitrophenyl-DL-methionine, 2,3-dimercaptosuccinic acid, and 2-mercaptosuccinic acid, were tested for their ability to promote direct electrochemistry of cyt. c' at a gold electrode. These twelve compounds were chosen on the basis of discussion by Allen et al.,[8] but the first six of compounds showed no promoter activity and the following five of compounds yielded extremely poor voltammetric responses. Only a compound, 2-mercaptosuccinic acid, promoted the electrochemistry of cyt. c'. Figure 1 shows a typical cyclic voltammogram of cyt. c'. A well-defined redox wave was observed on the voltammogram with -50 mV vs. NHE of cathodic peak potential and +50 mV vs. NHE of anodic one. The formal redox potential estimated from the midpoint between the cathodic and anodic peak potentials was 0 mV vs. NHE at pH 8.0, which agreed well with the value reported by potentiometry.[9] The peak currents increased linearly with increasing concentration of cyt. c' (50 - 300 μM) and square root of potential sweep rate (2 - 100 mV/s), but the intercepts did not coincide with the origin. In addition, the redox wave of the protein reappeared in a buffer solution at the same electrode after recording the first cyclic voltammogram in the protein solution followed by gentle washing, while the wave disappeared after vigorous washing. These results might indicate that cyt. c' was weakly adsorbed onto the surface-modified electrode through some interactions between the promoter and cyt. c'. Table 1 shows potential differences between anodic and cathodic peaks for cyt. c' obtained at surface-modified electrodes, together with those for cyt's c_2 and c. Table 1 also

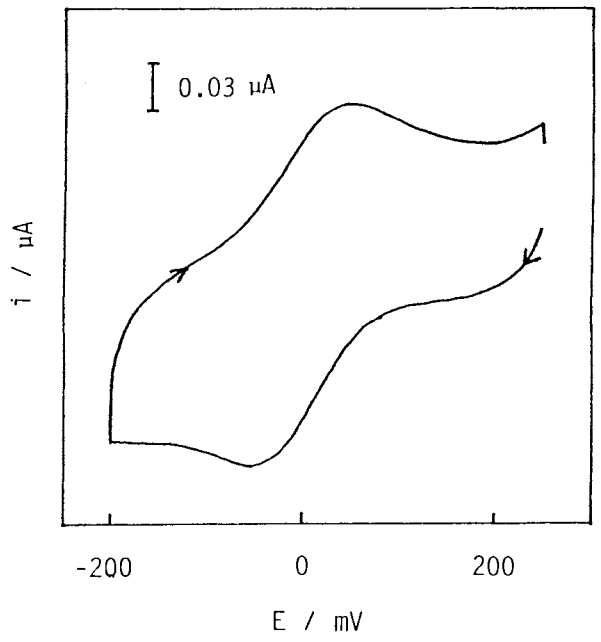

Fig. 1. Typical cyclic voltammogram of cyt. c' at 2-mercaptosuccinate-modified gold electrode. Concentration of cyt. c' was 200 μM in 0.1 M Tris buffer (pH 8.0). Potential sweep rate was 5 mV/s.

Table 1. Potential difference between anodic and cathodic peaks

Modifier (pK)	Cytochrome (pI)		
	c' (Rs. rubrum) (5.6(ox.))	c_2 (Rs. rubrum) (6.2)	c (horse heart) (10.5)
4-Mercaptopyridine (4.9)	——— a)	75 mV	70 mV
2,3-Dimercaptosuccinate (3.49)	160 mV	90 mV	170 mV
2-Mercaptosuccinate (3.28)	100 mV	85 mV	>300 mV

a) No redox waves could be observed on cyclic voltammogram. Concentration of cyt's was 200 μM in 0.1 M Tris buffer (pH 8.0), and potential sweep rate was 5 mV/s.

shows the isoelectric points (pI) of cyt's and the dissociation constants (pK) of modifiers. As proposed earlier,[8] an effective promoter should have binding groups capable of promoting weak adsorption of the protein, and the binding group seems to be anionic carboxylate or neutral pyridic nitrogen under the present condition (pH 8.0). On the other hand, cyt's c' and c_2 show the similar acidic pI values and therefore the molecular surfaces are totally negatively charged, while the surface of cyt. c is positively charged. These promoters should, therefore, give the similar voltammetric behavior for cyt's c' and c_2, if the interactions between the promoters and the proteins are restricted to be only electrostatic over whole surface of the proteins. The results, against the expectation, showed the better similarities especially on the redox behavior at 4-mercaptopyridine-modified electrode between cyt's c_2 and c rather than those between cyt's c' and c_2. These results might indicate that the interactions between the promoters and cyt's were not extended over whole surface of the proteins and were limited to a local domain of the surface. It is known that the positively charged lysine residues around the heme crevice of cyt. c are necessary for formation of protein-protein complexes *in vivo* with cyt. oxidase and other enzymes.[10-13] The interactions between the promoters and the proteins are, therefore, suggested to be through hydrogen bond and/or salt bridge formation between the anionic carboxylate or neutral pyridic nitrogen and the protonated lysine residues (pK = 10.53) around the heme crevice. This suggestion was supported by X-ray crystallographic studies on cyt's c,[14] c_2,[15] and c',[6] in which the distribution of lysine residues on cyt's c and c_2 was shown to almost be homologous whereas that on cyt's c and c' being hardly homologous. Both the peak separation (about 90 - 120 mV) and the current ratio of anodic peak to cathodic one (about 0.8 - 1.0) were close to those corresponding to a quasi-reversible one-electron transfer. The diffusion coefficient and the

heterogeneous electron transfer rate constant were estimated to almost be the order of 10^{-6} cm^2/s and 10^{-4} cm/s at different sweep rates and concentrations, respectively. The values indicate that the electron transfer between cyt. c' and the surface-modified electrode was moderately rapid, although none were more rapid than those for cyt. c_2 redox system.[3] In conclusion, the direct electrochemistry of cyt. c' using a surface-modified electrode would promise to examine the reaction kinetics on the promoter-protein complex as a model of the protein-protein complexes *in vivo*. However, it is not possible from the limited data to elucidate the interactions, and further detailed experiments on the effect of pH (especially around the isoelectric point) have become of major interest.

References

1) T. Yagi, H. Inokuchi, and K. Kimura, Acc. Chem. Res., 16, 2 (1983).
2) I. Taniguchi, Denki Kagaku, 56, 158 (1988) in Japanese.
3) T. Erabi, H. Yuasa, K. Hirata, and M. Wada, Chem. Express, 4, 809 (1989).
4) T. Erabi, S. Nakamoto, H. Yuasa, H. Tohda, S. Hayase, and M. Wada, Chem. Express, 7, 529 (1992).
5) R. G. Bartsch, "The Photosynthetic Bacteria," ed by R. K. Clayton and W. R. Sistrom, Plenum Press, New York (1978), p. 265.
6) B. C. Finzel, P. C. Weber, K. D. Hardman, and F. R. Salemme, J. Mol. Biol., 186, 627 (1985).
7) R. G. Bartsch, T. Kakuno, T. Horio, and M. D. Kamen, J. Biol. Chem., 246, 4489 (1971).
8) P. M. Allen, H. A. O. Hill, and N. J. Walton, J. Electroanal. Chem., 178, 69 (1984).
9) T. Kakuno, K. Hosoi, T. Higuti, and T. Horio, J. Biochem., 74, 1193 (1973).
10) S. Ferguson-Miller, D. L. Brautigan, and E. Margoliash, J. Biol. Chem., 253, 149 (1978).
11) N. Osheroff, D. L. Brautigan, and E. Margoliash, J. Biol. Chem., 255, 8245 (1980).
12) S. H. Speck, W. H. Koppenol. J. K. Dethmers, N. Osheroff, E. Margoliash, and K. V. Rajagopalan, J. Biol. Chem., 256, 7394 (1981).
13) E. C. I. Veerman, J. Wilms, H. L. Dekker, A. O. Muijsers, K. J. H. Van Buuren, B. F. Van Gelder, N. Osheroff, S. H. Speck, and E. Margoliash, J. Biol. Chem., 258, 5739 (1983).
14) R. D. Dickerson, T. Takano, D. Eisenberg, O. B. Kallai, L. Samson, A. Cooper, and E. Margoliash, J. Biol. Chem., 246, 1511 (1971).
15) F. R. Salemme, S. T. Freer, Ng. H. Xuong, R. A. Alden, and J. Kraut, J. Biol. Chem., 248, 3910 (1973).

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