Vol. 145, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 29, 1987 Pages 619-624

DIRECT ELECTRON TRANSFER REACTIONS OF CYTOCHROME c_{553} FROM DESULFOVIBRIO VULGARIS HILDENBOROUGH AT INDIUM OXIDE ELECTRODES

Kent B. Koller[ab], Fred M. Hawkridge[a*], Guy Fauque[cd], and Jean LeGall[cd*]

[a] Department of Chemistry, Virginia Commonwealth University, Box 2006, Richmond, Virginia 23284

[b] Naval Research Laboratory, Code 6170, 4555 Overlook Ave., S. W., Washington, D.C. 20375

[c] Department of Biochemistry, University of Georgia, Athens, Georgia 30602

[d] C.E.N. Cadarache, A. R. B. S. Section Enzymologie et Biochimie Bacterienne, 13108 Saint Paul lez, Durance, FRANCE

Received April 6, 1987

The direct, heterogeneous, electron transfer reactions of cytochrome c_{553} from Desulfovibrio vulgaris Hildenborough have been studied at indium oxide optically transparent electrodes. These reactions have been studied using cyclic voltammetry and derivative cyclic voltabsorptometry and the kinetics of heterogeneous electron transfer is quasi-reversible. The thermodynamics and kinetics of electron transfer by this molecule can be studied at this electrode surface without the need for surface modification or the addition of surface promoters or mediators.

The sulfate-reducing bacteria constitute a metabolically specialized group of strict anaerobic microorganisms that carry out the dissimilatory reduction of sulfate and other anions of sulfur (1,2). The sulfate-reducers of the genus Desulfovibrio contain several kinds of c-type cytochromes and a classification based on the number of hemes per molecule has been recently proposed (3).

Three classes can be distinguished: monoheme, tetraheme and octaheme cytochromes c. Tetraheme and octaheme cytochromes c_3

* Authors to whom correspondence should be addressed.

0006-291X/87 $1.50

have very low redox potential with two histidine residues as iron axial ligands and tetraheme cytochromes c_3 are the only hemoproteins present in all Desulfovibrio species so far isolated (1,3). The cytochrome c_{553} belongs to the class of monohemic cytochromes; it is a low molecular weight protein with histidine and methionine as fifth and sixth axial heme iron ligands. Cytochrome c_{553} has been isolated and characterized in Desulfovibrio (D.) vulgaris strains Hildenborough and Miyazaki (4,5), D. desulfuricans strains Berre-Eau (6) and Berre-Sol (G. Fauque, I. Moura, J. J. G. Moura, A. V. Xavier and J. LeGall, unpublished results). Cytochrome $c_{553(550)}$ is another monohemic protein found in D. desulfuricans strain Norway 4 (7) and D. baculatus DSM 1743 (8). Desulfovibrio monoheme cytochromes have midpoint redox potentials between 0 and 50 mV vs NHE (9,10) which are low in comparison with other methionine-histidine ligated monohemic cytochromes (11).

We report here the results of a study of the electron transfer reactions of cytochrome c_{553} from D. vulgaris Hildenborough at indium oxide electrodes. No mediators, promoters or electrode modifiers were used in this work. These electron transfer reactions have been studied by cyclic voltammetry (CV) (12) and derivative cyclic voltabsorptometry (DCVA) (13). The rate of heterogeneous electron transfer is quasi-reversible and the responses are stable for several hours.

PROCEDURES

Organism and Growth Conditions. D. vulgaris strain Hildenborough (NCIB 8303) was grown in the medium of Starkey (14) on lactate sulfate at 37°C and harvested as previously described (8).

Analytical Procedures. Absorption spectra were obtained on a Beckman Model 35 spectrophotometer. The purity of the cytochrome c_{553} was checked by analytical gel electrophoresis performed according to Davis (15) in 7% polyacrylamide gel with Tris-(hydroxymethyl amino methane hydrochloride) (Tris-HCl) glycine buffer at pH 8.9.

Purification of D. vulgaris Cytochrome c_{553}. All purification steps were performed at 4°C using potassium phosphate and Tris-HCl buffers, pH 7.6, of appropriate molarity. During the purification a purity coefficient will be defined as $[A_{553}(red)-A_{570}(red)]/A_{280}(oxid)$. D. vulgaris strain Hildenborough cytochrome c_{553} was purified in four steps following a different procedure than that previously described (4). To 600 g wet weight cells, 700 ml of 10 mM Tris-HCl buffer containing a few desoxyribonuclease crystals were added. The cell suspension was treated twice in a French pressure cell and the resulting extract was centrifuged at 12,000 rpm for 1 hour. The crude extract was passed over a DEAE Bio-Gel A column (28x4.5 cm) equilibrated with 10 mM Tris-HCl. The fraction not adsorbed on this column contained both cytochrome c_{553} and tetrahemic cytochrome c_3 and was loaded on a CM Bio-Gel A column (23x4.5 cm) equilibrated with 10 mM Tris-HCl. The cytochrome fraction eluted with a linear gradient of Tris-HCl (500 ml of 10 mM and 500 ml of 250 mM) and it exhibited a purity coefficient of 0.19. The cytochrome fraction was adsorbed onto a hydroxylapatite (Bio-Gel HTP) column (17x4.5 cm) equilibrated with 250 mM Tris-HCl. During the decreasing linear gradient of Tris-HCl (300 ml of 250 mM and 300 ml of 10 mM) the cytochrome c_{553} was eluted (at ≈100 mM Tris-HCl) and separated from tetraheme cytochrome c_3 (which eluted only after a linear gradient of potassium phosphate buffer). The cytochrome c_{553}, with a purity index of 0.98, was dialyzed overnight against 10 l of 5 mM Tris-HCl and concentrated in a ultrafilter Diaflo Amicon with a YM-5 membrane. In the last step of purification the cytochrome c_{553} was passed over a second CM Bio-Gel A column (20x4.5 cm) equilibrated with 10 mM Tris-HCl and eluted with a linear Tris-HCl gradient (400 ml of 10 mM and 400 ml of 250 mM). By this procedure 48 mg of pure D. vulgaris strain Hildenborough cytochrome c_{553} were obtained with a purity coefficient of 1.16. This hemoprotein is partly reduced by sodium ascorbate and completely by sodium dithionite.

Electrochemical and Optical Instrumentation and Procedures. The electrochemical and optical instrumentation used in this work have been previously described (16). The procedures for acquiring the CV and DCVA results have also been described (13,16). Procedures for pretreatment of the indium oxide electrodes (PPG Industries) have been described (17) and all potentials reported here are referenced to the Normal Hydrogen Electrode (NHE).

RESULTS AND DISCUSSION

Figure 1 shows background subtracted cyclic voltammograms of D. vulgaris Hildenborough cytochrome c_{553} reacting at a clean indium oxide electrode in the absence of mediators, promoters or chemical modifiers of the electrode surface. Figure 2 shows a plot of peak current versus the square root of the scan rate that is linear and consistent with a reversible electrode reaction. The diffusion coefficient of D. vulgaris cytochrome c_{553} calculated from the slope of this line (18) is 1.6×10^{-6} cm^2/s. The

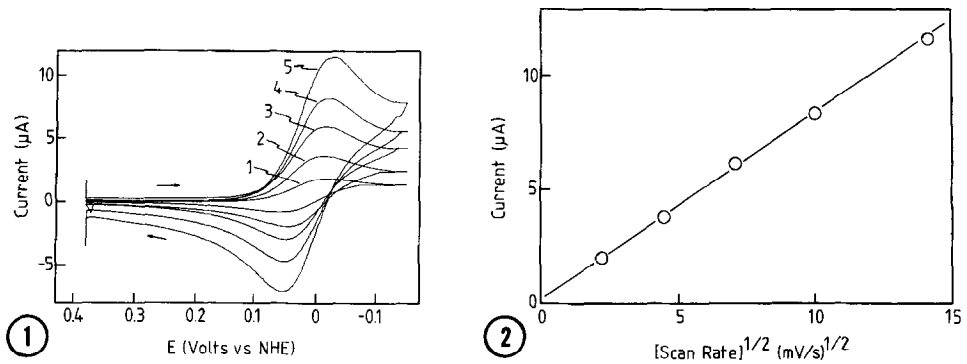

Figure 1. Cyclic Voltammetry of D. vulgaris Hildenborough Cytochrome c_{553} at an Indium Oxide Electrode. Solution contained 60 μM cytochrome c_{553}, 0.01 M Tris-HCl, pH 7.6. Electrode area 1.23 cm^2, scan rates in mV/s: (1) 5, (2) 20, (3) 50, (4) 100, and (5) 200.

Figure 2. Plot of Peak Current versus the Square Root of the Scan Rate. Each point is the difference in the average of three experiments with cytochrome c_{553} present and three experiments with buffer alone. Values of peak current in μA and (scan rate)$^{1/2}$ in (mV/s)$^{1/2}$ are: 2.0, 2.24; 3.8, 4.47; 6.1, 7.07; 8.3, 10.0; and 11.6, 14.1. Intercept = 0.21 μA, slope = 0.807, r = 0.9996.

formal potential calculated from these CVs using the equation $(E_{p,c} + E_{p,a})/2$ is 0.018(±0.001) V vs NHE, in excellent agreement with the previously reported value of 0.02(±0.01) V vs NHE (9,19). These data were also used to determine the formal heterogeneous electron transfer rate constant for the reaction of cytochrome c_{553} at indium oxide electrodes (12), 7.5(±1.0) x 10^{-3} cm/s.

Figure 3 shows a slow scan DCVA for the reaction of D. vulgaris cytochrome c_{553} at an indium oxide optically transparent (OTE) electrode in oxygen saturated buffer. On the time scale of this experiment there is no evidence that the electrochemically formed ferro-cytochrome c_{553} is oxidized by the presence of dioxygen. The ratio of the peak cathodic to peak anodic optical response is 1.00(±0.05).

Large and interesting differences in the redox properties exist between the monohemic and plurihemic cytochromes c from Desulfovibrio. The tetrahemic cytochrome c_3 from D. gigas has

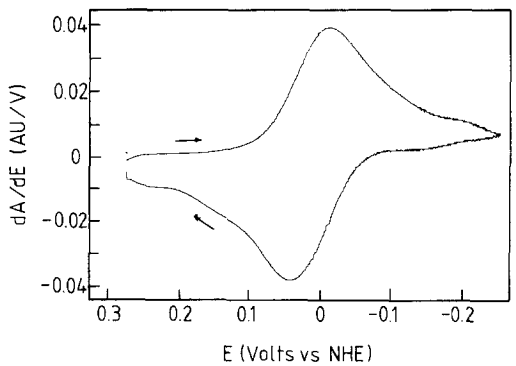

Figure 3. Derivative Cyclic Voltabsorptometry of D. vulgaris Hildenborough Cytochrome c_{553} at an Indium Oxide Electrode. Same conditions as described in Figure 1, scan rate is 5.0 mV/s, solution is oxygen saturated.

redox potentials of -235, -235, -306 and -315 mV (20), from D. vulgaris Hildenborough the values are -284, -310, -319 and -324 mV (21) and from D. desulfuricans Norway 4 the values are -125, -125, -305 and -325 mV (22).

The physiological function of Desulfovibrio monohemic cytochromes c_{553} and $c_{553(550)}$ remains unknown; this is also the case for other electron carriers with relatively high redox potentials (e.g., around 0 mV) such as rubredoxin and desulforedoxin (23). Their participation in metabolic pathways involved in dissimilatory sulfate reduction is difficult to rationalize based on their high formal potentials (3).

The results presented here demonstrate that the thermodynamics and kinetics of electron transfer by D. vulgaris Hildenborough cytochrome c_{553} can be directly studied using electrochemical and optical methods at indium oxide electrodes.

ACKNOWLEDGEMENTS

We are indebted to M. Scandellari and R. Bourrelli for growing the bacteria used in the present study and we thank B. Prickril for his technical help during the purification. This research work was supported by grants from the National Science

Foundation DMB-8602789 (J. LeGall), CHE-8520270 (F. M. Hawkridge) and from the NSF/CNRS Fellowship Program (G. Fauque).

REFERENCES

1. Postgate, J. R. (1984) The Sulphate-Reducing Bacteria, 2nd ed., Cambridge University Press, Cambridge, pp. 208.
2. Widdel, F. (1987) Microbiology and Ecology of Sulfate and Sulfur-Reducing Bacteria. In: Biology of Anaerobic Microorganisms (A. J. B. Zehnder, ed.) John Wiley & Sons, New York, in press.
3. LeGall, J. and Fauque, G. (1987) Dissimilatory Reduction of Sulfur Compounds. In: Biology of Anaerobic Microorganisms (A. J. Zehnder, ed.) John Wiley & Sons, New York, in press.
4. LeGall, J. and Bruschi-Heriaud, M. (1968). Structure and Function of Cytochromes. pp. 467-470, University Park Press, College Park, Maryland.
5. Yagi, T. (1979) Biochim. Biophys. Acta 548, 96-105.
6. Moura, I., Fauque, G., LeGall, J., Xavier, A. V. and Moura, J. J. G. (1987) Eur. J. Biochem. 162, 547-554.
7. Fauque, G., Bruschi, M. and LeGall, J. (1979) Biochem. Biophys. Res. Commun. 86, 1020-1029.
8. Fauque, G. (1979) These de Doctorat de Specialité, 121 pages, University of Aix-Marseille I, France.
9. Bertrand, P., Bruschi, M., Denis, M., Gayda, J. P. and Manca, F. (1982) Biochem. Biophys. Res. Commun. 106, 756-760.
10. Bianco, P., Haladjian, J., Loufti, M. and Bruschi, M. (1983) Biochem. Biophys. Res. Commun. 113 526-530.
11. Moore, G. R., Pettigrew, G. W., Pitt, R. C. and Williams, R. J. P. (1980) Biochim. Biophys. Acta 590, 261-271.
12. Nicholson, R. S. (1965) Anal. Chem. 37, 1351-1355.
13. Bancroft, E. E., Sidwell, J. S. and Blount, H. N. (1981) Anal. Chem. 53, 1390-1394.
14. Starkey, R. L. (1938) Arch. Microbiol. 9, 268-304.
15. Davis, B. J. (1964) Ann. N. Y. Acad. Sci. 121, 404-427.
16. Koller, K. B. and Hawkridge, F. M. (1985) J. Am. Chem. Soc. 107, 7412-7417.
17. Bowden, E. F., Hawkridge, F. M., Chlebowski, J. F., Bancroft, E. E., Thorpe, C. and Blount, H. N. (1982) J. Am. Chem. Soc. 104, 7641-7644.
18. Bard, A. J. and Faulkner, L. R. (1980) Electrochemical Methods, John Wiley & Sons, New York, p. 218.
19. Bianco, P., Haladjian, J., Pilard, R. and Bruschi, M. (1982) J. Electroanal. Chem. 136, 291-299.
20. Xavier, A. V., Moura, J. J. G., LeGall, J. and DerVartanian, D. V. (1979) Biochimie 61, 689-695.
21. DerVartanian, D. V., Xavier, A. V. and LeGall, J. (1978) Biochimie 60, 321-325.
22. Cammack, R., Fauque, G., Moura, J. J. G. and LeGall, J. (1984) Biochim. Biophys. Acta 784, 68-74.
23. Moura, I., Moura, J. J. G., Santos, M. H., Xavier, A. V. and LeGall, J. (1979) FEBS Lett. 107, 419-421.