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TETRAHEMOPROTEIN, CYTOCHROME c_3 AS AN ORGANIC CONDUCTIVE MATERIAL

Kenji ICHIMURA, Keisaku KIMURA,* Yūsuke NAKAHARA,†
Tatsuhiko YAGI,†† and Hiroo INOKUCHI

Institute for Molecular Science, Okazaki, Aichi 444, † Department of Electric Engineering, Technical College of Miyakonojo, Miyazaki 885, and †† Department of Chemistry, Shizuoka University, Shizuoka 422

The electrical conductivity of a cytochrome c_3 anhydrous film was measured in the region between 269 and 300 K under high-pressure hydrogen at 1000 kPa. The minimum value of the electrical resistivity of cytochrome c_3 reached to the order of 1×10^{-3} Ωcm at 292 K.

Cytochrome c_3, an electron carrier in the respiratory chain of Desulfovibrio vulgaris, is known to have four hemes from elemental analysis and spectroscopy.[1)] It is the only one protein which is subject to reversible electrochemical reduction and oxidation in the native state. The electron transfer occurs via 1 electron - 4 step process and the rate of the transfer is very large as compared to the inorganic ions. The rate constant at solution electrode interface exceeds 0.1 cm/s. The redox potential is very low as -0.287 V.[2,3)]

Cytochrome c_3 is reduced with molecular hydrogen by the catalysis of hydrogenase [hydrogen: ferricytochrome c_3 oxidoreductase, EC 1.12.2.1].[4)] The reaction is reversible and is expressed as following equation:

$$\text{Ferricytochrome } c_3\ (4Fe^{3+}) + 2H_2 \xrightleftharpoons[(4e^-)]{H_2ase} \text{Ferrocytochrome } c_3\ (4Fe^{2+}) + 4H^+. \qquad (1)$$

Hydrogenase retains its activity even in the anhydrous state as reported previously.[5)] Therefore we could prepare reduced cytochrome c_3 in the solid state for the electrical conductivity measurement.

Cytochrome c_3 is an extraordinarily good conductor among biological materials. We have already found that the electrical conductivity of cytochrome c_3 in anhydrous state was 60 Ωcm under 100 kPa hydrogen pressure and 8 Ωcm under 200 kPa hydrogen pressure. The conductivity increased with proportional to a cubic in hydrogen pressure.[6)] It is thus very interesting to measure the conductivity at a higher pressure. In the present work the pressure of hydrogen was expanded from 200 kPa to 1000 kPa.

Cytochrome c_3 and hydrogenase were highly purified from sulfate-reducing bacteria, D. vulgaris, Miyazaki, following the reported procedure.[1,7)] An anhydrous film of cytochrome c_3, containing a trace of hydrogenase, was prepared on a quartz plate whose dimension was 5 x 10 x 0.5 mm^3, by the same method as described previously.[6)] A high-pressure cell and measurement system, the pressure range from 10^{-3} Pa up to 7500 kPa, was designed and constructed. The cell, in which the quartz plate was located, was evacuated at 10^{-3} Pa for 6 hours and then hydrogen gas (>99.99999 % purity) was introduced into the cell at 300 K. Under these conditions ferricytochrome c_3 was reduced to ferro-form in 10 - 15 days.

After reduction of a cytochrome c_3 anhydrous film, the conductivity was measured. The ohmic law was found to hold under an applied potential between 0.1 mV and 1 V. In this work, we observed the current continuously under the applied potential of 0.1 mV in the range between 269 and 300 K. A reliable result was obtained at the current region above 10^{-9} A, in which the noise was negligible. The rate of temperature rise and fall was controlled to be 0.1 K/min by the use of a manganin heater and liquid nitrogen. The temperature of the sample was monitored precisely by a copper-constantan thermocouple in contact with the sample plate.

Figure 1 shows the logarithm of the current vs. the reciprocal of the absolute temperature for the anhydrous film of cytochrome c_3 at 1000 kPa hydrogen pressure. The temperature dependence of resistivity was reversible and the reproducibility was good in the whole region of temperature measured for several runs. The identical result was obtained for two samples with different thickness. Comparing with the peak at the lower hydrogen pressure, a very sharp peak appeared at T_m = 292 K and the value of current was 1.3 x 10^{-6} A. In this work, a cubic law mentioned above[6)] also holds between the hydrogen pressure and the electrical current. The region below T_m represents a semiconductive behavior of ferrocytochrome c_3 and the activation energy was evaluated to be 2.1 eV. The observed value of the activation energy agreed well with that reported in the previous paper.[6)] Above T_m, the temperature dependence of the current is thought to be for the mixed state of ferri- and ferrocytochrome c_3.

Assuming the same molar extinction coefficient both for solution and solid state, the thickness of a film, d, was calculated to be 82 nm.[6)] The electrical resistivity

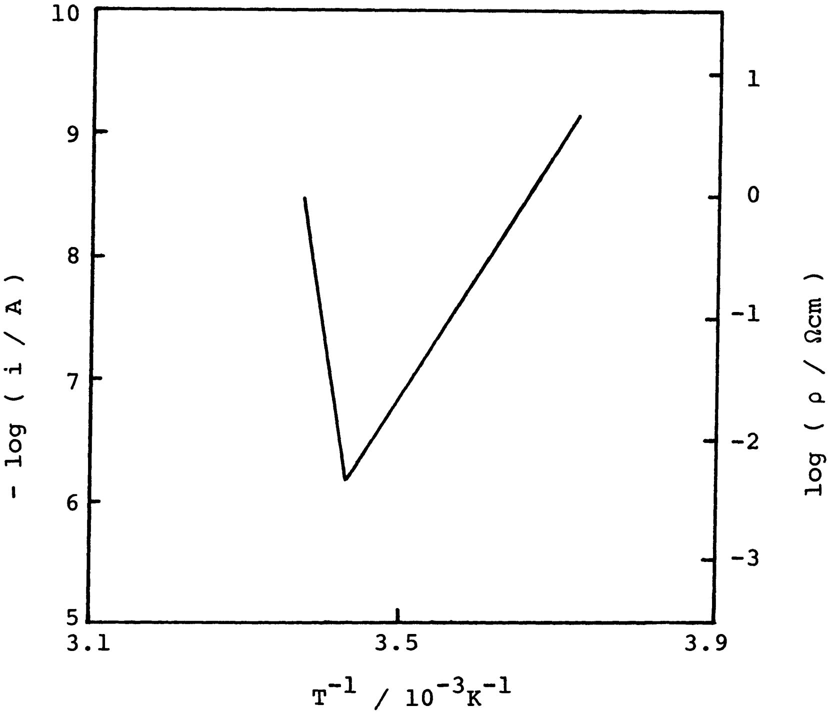

Fig.1. The electrical conductivity of a cytochrome c_3 anhydrous film, current vs. temperature at 1000 kPa hydrogen.

ρ at T_m is given by the use of the following values, thickness d = 82 nm, distance between electrodes l = 1.0 mm, length of the film L = 5.0 mm, supplied voltage E = 0.1 mV, and the observed current $i = 1.3 \times 10^{-6}$ A : $\rho = (dL/l)(E/i) = 3.2 \times 10^{-3}$ Ωcm. The resistivity at the extreme is only 3×10^{-3} Ωcm which is extraordinarily small as a protein. The value could be comparable to those of organic metals ; for instance TMTTF-TCNQ 1:1 salt ($\rho = 1 - 3.3 \times 10^{-3}$ Ωcm at room temperature).[8)]

The oxidoreduction of cytochrome c_3 is coupled with the electron transfer. The analysis of the reaction order may give an insight to the conduction mechanism. The order with respect to hydrogen pressure is nearly unity. On the other hand, the hydrogen order for the equilibrium constant of Eq. 1 determined by conductivity measurement was 3. This discrepancy suggests that there is not direct relation between the conductivity and reaction. However, the fact that the resistivity of a cytochrome c_3 film decreases with an increase of hydrogen pressure indicates a carrier generation and/or mobility relate to the equilibrium condition.

Recently, the molecular structure was determined at 2.5 Å resolution by the X-ray diffraction method.[9)] It was found that four hemes are exposed to the surface of a molecule which might be the cause of intermolecular heme-heme interaction in the solid state. The overall dimension of cytochrome c_3 is approximately 33 x 39 x 34

Å^3. The distances among the central iron atoms in hemes range from 11.3 to 18.1 Å. All heme groups find aromatic residues in proximity. That is, all phenylalanine and tyrosine residues are closed near the hemes, the plane of these aromatic side groups except tyrosine 65 being parrallel to the imidazole ring of the coordinated histidine. Those aromatic residues must intervene the electron transfer and also the magnetic interaction between hemes. Studies on Moessbauer and ESR clarified the intermolecular heme-heme interaction also exists in the solid state, causing the delocalization of the π-electrons among hemes. The electron transfer seems to occur through the π-electron systems of aromatic residues around hemes. The conformation of this molecule is essential for the high electric conductivity.

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