CHEMISTRY LETTERS, pp. 877-880, 1979. Published by the Chemical Society of Japan

ELECTRICAL CONDUCTIVITY OF SOLID STATE PROTEINS: SIMPLE PROTEINS AND CYTOCHROME c_3 AS ANHYDROUS FILM

Yūsuke NAKAHARA, Keisaku KIMURA, Hiroo INOKUCHI, and Tatsuhiko YAGI

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

The difference in electrical resistivity between hemoproteins and simple proteins was reported. The resistivities of mono-hemoproteins were in the range of 10^9-10^{11} Ωcm at 303 K, whereas that of ferrocytochrome c_3 was extremely low, and possessed an anomalous temperature dependence. The resistivity of cytochrome c_3 was as low as 57 Ωcm at 268 K. The inclination of the temperature dependence corresponded to the value of 3.7×10^5 J·mol^{-1}. Above this temperature, the resistivity increased.

In the extensive studies by Eley's school[1-3], although no clear differences were observed in the conductivities between so-called electron transfer proteins and other proteins, the semiconductive behavior of protein has been found. However, the conduction mechanism has not yet been clarified. We have found[4] that there was a large difference in the resistivity between ferricytochrome c and ferrocytochrome c which was attributable to the electronic state of central metal ion. It is thus important to compare the electrical conductivity of cytochrome c with other hemoproteins and simple proteins having the same molecular weights in order to reveal the effect of heme on the conduction mechanism.

Cytochrome c is a globular hemoprotein with a molecular weight (M_r) of about 12500. As the representatives of globular simple proteins, we chose lysozyme (M_r=14600)[5], ribonuclease (M_r=13700)[5], and trypsin (M_r=23800)[5]. Myoglobin (M_r=17000)[5], was also chosen as a member of hemoprotein though it is not an electron transfer protein. As a multihemoprotein, the electrical conductivity of cytochrome c_3 (M_r=14000)[6] was measured as well.

In the course of the present study, special attention was paid in preparing samples not to denature proteins, since the characteristics of each protein might be lost upon denaturation. The film casting method to prepare sample films[4] is advantageous as compared with earlier methods[1-3], because the method enables us to prepare the sample at physiological conditions. This method also provides a very thin film, 100-1000 nm in thickness, so that a trace of water is easily removed. These and other advantages were discussed in another paper[7].

Cytochrome c_3 is an electron carrier in a respiratory chain of Desulfovibrio vulgaris and has 4 hemes in a molecule. The findings by NMR[8] and Mössbauer spectroscopies[9] suggest that the heme-heme interaction in cytochrome c_3 molecules is

fairly strong as a biomolecule. Cytochrome c_3 undergoes reversible oxidoreduction by a catalytic action of hydrogenase even in the anhydrous film state[10].

$$\text{ferricytochrome } c_3\ (Fe^{3+}) + 2H_2 \rightleftharpoons \text{ferrocytochrome } c_3\ (F^{2+}) + 4H^+$$

Therefore, the conductivity of cytochrome c_3 of ferro-form can be measured after reducing the ferri-form in film state by introducing molecular hydrogen. Recently, the reversible electrochemical electron transfer of cytochrome c_3 was observed in a phosphate buffer solution[11]. It is noticeable that the electron transfer rate constant at electrode-solution interface exceeds 0.1 cm/s, one of the largest values ever obtained among inorganic and organic substances. The heme-heme interaction in cytochrome c_3 may be also reflected in the conductivity of a cytochrome c_3 film if the heme part contributes to the conductivity mechanism.

Cytochrome c_3 and hydrogenase were isolated from D. vulgaris, Miyazaki, and highly purified as reported previously[6,12]. Cytochrome c_3 solution was then dialyzed thoroughly against distilled water. Lysozyme, ribonuclease, trypsin and myoglobin were the products of Boehringer Mannheim, GmbH and used without further purifications. Lyophilized powders of 3 mg cytochrome c_3 (210 nmol) and 13.2 units of hydrogenase (0.24 nmol) were dissolved in 6 ml distilled water. After the sample solution was degassed under reduced pressure, a cytochrome c_3 film was deposited on a quartz plate from the solution by means of evaporation. The details of film preparation have already been reported[4]. The film thickness was determined by a surface roughness tester, Dektak, product of Sloan Ltd. Films of lysozyme, ribonuclease, trypsin and myoglobin were also prepared on quartz plates by the same method as in the cytochrome c_3.

The sample film was then placed in a conductivity-measurement chamber connected with a vacuum system and with a gas inlet system. After one hour heating at 323 K in a dry hydrogen atmosphere, it was evacuated at 10^{-4} Pa for one hour in the same temperature in order to remove a trace of water included in a sample. In order to obtain a ferrocytochrome c_3 film, a dry hydrogen gas was introduced to the chamber and settled for 3-5 days. The d.c. conductivity of a ferrocytochrome c_3 film was measured in an atmosphere of dry hydrogen gas of 94.7 kPa under 100 V applied voltage. The distance between electrodes was 2 mm. The temperature dependence of the conductivity was measured with successively raising the temperature at a rate of 0.1 - 0.03 K/s after the sample was held at 203 K for 5 minutes. The conductivity of ferricytochrome c_3, on the other hand, was measured in an atmosphere of 13.3 kPa nitrogen gas. The conductivities of lysozyme, ribonuclease, trypsin and myoglobin were also measured in a dry nitrogen atmosphere in the range from 283 to 343 K, with 500 V applied voltage after heat and evacuation cycles.

It has been found that the current-voltage relation strongly depended on the amount of water contained in a film as already reported[4]. The ohmic law was held in a well dried sample which supported the conduction carrier was electronic. The rise times of the transient currents against the step change of the input voltage were ten or more minutes for a wet sample and a few seconds for a well dried sample at room temperature irrespective of sample species. A steady state current was examined for one day in a ferrocytochrome c_3 film. These findings also support the electronic conduction.

The electrical resistivity of simple proteins was so high that the electric current was not observed in the range from 283 to 343 K: The resistivities of these proteins were higher than 10^{14} Ωcm. Figure 1 shows the observed curve of the temperature dependence of the current through a ferrocytochrome c_3 film together with that of ferricytochrome c_3. The relation of the logarithm of the resistivity against the reciprocal of the absolute temperature is reproducible in the ferro- and ferri-forms. As comparison with other organic solids, the V shape dependence of resistivity on temperature seems very unusual. It may be correct to say that the region below T_M of line (1) represents a semiconductive nature of ferrocytochrome c_3. In the temperature region above T_M, it is suggested that there will be a different mechanism from an ordinary conduction. Cytochrome c_3 is known to be in equilibrium between ferri- and ferro-forms at a given temperature under H_2 in the presence of hydrogenase, so that both forms of cytochrome c_3 mix each other in region (a), that is to say, ferri- and ferro-forms coexist in this region. This coexistence was confirmed and analyzed in another report[7].

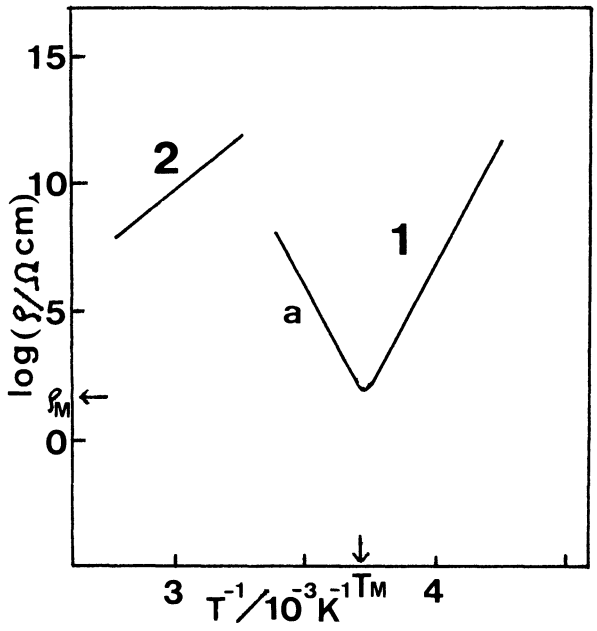

Fig. 1 Temperature dependence of the resistivity of (1) ferrocytochrome c_3, and (2) ferricytochrome c_3.

Table 1 collects the electrical conductivity data of several protein solids. The ferri-forms of both cytochromes have a similar resistivity in magnitude at room temperature. On the other hand, very drastic difference appeared in the resistivity of the ferro-form. At 268 K, the electrical resistivity of ferrocytochrome c_3 was only 57 Ωcm, which corresponded to that of Ge.

The contribution of heme to the electrical conduction of biological materials is clear because the polypeptide chain itself is an electrical insulator whose resistivity exceeds 10^{14} Ωcm. On the other hand, the resistivity of one hemoprotein keeps about 10^9-10^{11} Ωcm. Eley and Spivey[2], in their pioneer work of 1960, could not find the difference in conductivity of hemoprotein from that of simple proteins. The reason may be accounted for by the inadequacy of sample preparations, i.e., application of high temperature, and high pressure which may lead to denaturation of protein.

Taking into account of the size of porphyrin, 1.3 nm in diameter, the distance is small enough to cause the overlapping of π electrons. Electrochemical measurement of the reduction of cytochrome c_3 on a mercury electrode[11] also suggests us that some parts of hemes are exposed to the protein surface and/or the electrons tunnel through a peptide residue.

The present study implies not only the importance of cytochromes existing in ferri- and ferro-forms in biological systems but also an expectation to have new

conductive materials in the field of biological substances.

This research was supported in part by a Grant (111911, 1976 and 211112, 1977) from the Ministry of Education of Japan.

Table 1

Materials	Activation energy $\Delta E/J \cdot mol^{-1}$	Resistivity $\rho(303K)/\Omega$ cm	ρ_0/Ω cm	Reference
Lysozyme	——	$>10^{14}$	——	Present study
Ribonuclease	——	$>5\times10^{14}$	——	Present study
Trypsin	——	$>10^{14}$	——	Present study
Myoglobin	1.5×10^{4}	3.6×10^{10}	1.2×10^{8}	Present study
Cytochrome c				
oxid	1.3×10^{5}	6.1×10^{16} a)	1.6×10^{-5}	2
oxid	1.1×10^{5} (343-373K)	4.8×10^{15} a)	2.5×10^{-3}	13
	5.8×10^{4} (313-343K)			13
oxid	5.8×10^{4} (323-358K)	3.1×10^{11} a)	4.0×10^{1}	4
red	5.8×10^{4} (283-333K)	3.1×10^{9}	4.0×10^{-1}	4
Cytochrome c_3				
oxid	1.6×10^{5} (293-343K)	2.3×10^{12}	5.4×10^{-16}	Present study
red	3.7×10^{5} (233-268K)	5.7×10^{1} b)	2.5×10^{-70}	Present study

a) The resistivity at 303 K by the extrapolation with ΔE and ρ_0

b) Value at 268 K (T_M)

References

1) M.H.Cardew and D.D.Eley, Discuss. Faraday Soc., 27, 115(1959).
2) D.D.Eley and D.I.Spivey, Trans. Faraday Soc., 56, 1432(1960).
3) D.D.Eley and W.P.Williams, Trans. Faraday Soc., 64, 1528(1968).
4) Y.Nakahara, K.Kimura and H.Inokuchi, Chem. Phys. Lett., 47, 251(1977).
5) "Outlines of Biochemistry", 3rd. Ed. by E.E.Conn and P.K.Stumpf, Sec. 8, John Wiley & Sons, Inc. (1972).
6) T.Yagi and K.Maruyama, Biochim. Biophys. Acta, 243, 214(1971).
7) K.Kimura, Y.Nakahara, T.Yagi and H.Inokuchi, J. Chem. Phys. 70, 3317(1979).
8) C.M.Dobson, N.J.Hoyle, C.F.Geraldes, P.E.Wright, R.J.P.Williams, M.Bruschi and J.LeGall, Nature, 249, 425(1974).
9) K.Ono, K.Kimura, T.Yagi and H.Inokuchi, J. Chem. Phys., 63, 1640(1975).
10) K.Kimura, A.Suzuki, H.Inokuchi and T.Yagi, Biochim. Biophys. Acta, 567, 96(1979).
11) K.Niki, T.Yagi, H.Inokuchi and K.Kimura, J. Electrochem. Soc., 124, 1889(1977).
12) T.Yagi, K.Kimura, H.Daidoji, F.Sakai, S.Tamura and H.Inokuchi, J. Biochem., 79, 661(1976).
13) P.Taylor, Discuss. Faraday Soc., 27, 239(1959).

(Received April 27, 1979)