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Self-reduction and Conductivities of Cytochromes

Kenji Ichimura [a] , Yusuke Nakahara [b] & Hiroo Inokuchi [c]

[a] Graduate School of Science and Technology, Kumamoto University, Kurokami, Kumamoto, Japan

[b] Department of Research and Development, Sunny Sealing Co., Ltd., Sibita, Miyakonojo, Miyazaki, Japan

[c] JAXA, Hamamatsu-cho, Minato-ku, Tokyo, Japan

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Self-reduction and Conductivities of Cytochromes

Kenji Ichimura
Graduate School of Science and Technology, Kumamoto University, Kurokami, Kumamoto, Japan

Yusuke Nakahara
Department of Research and Development, Sunny Sealing Co., Ltd., Sibita, Miyakonojo, Miyazaki, Japan

Hiroo Inokuchi
JAXA, Hamamatsu-cho, Minato-ku, Tokyo, Japan

A self-reduction of cytochrome c is found and depends on the pressure of hydrogen, pH, and sorts of buffers and salts. Cytochrome c_3 shows the two mechanisms such as the reduction of heme and hydrogen exposure effect.

Keywords: bio-organic conductors; bio-organic semiconductors; cytochromes; cytochrome c_3; hydrogen; self-reduction

INTRODUCTION

The conductivity of hemo-protein film is found to depend the redox states of the hemes [1,2]. While Ferri-cytochrome c_3 is an insulator, ferro-cytochrome c_3 is a semiconductor under the hydrogen pressure of less than around 1 MPa. The temperature dependence of the conductivity was presented as an unusual form of Z-type [3,4]; a semiconductive temperature dependence changes to an apparently metallic dependence, and then changes again to the semiconductive dependence.

The conductivities in the pressure range of more than 2 MPa were measured, and the temperature dependence of the conductivity of ∧Z-type was obtained. Moreover, marvelously metallic characteristics appeared under the higher hydrogen pressure range of $3 \sim 5$ MPa. This

Address correspondence to Kenji Ichimura, Graduate School of Science and Technology, Kumamoto University, Kurokami, Kumamoto 860-8555, Japan. E-mail: ichimura@gpo.kumamoto-u.ac.jp

work reveals the self-reduction of cytochrome c in the cast film and the water solution under hydrogen atmosphere by means of UV-visible-near infrared spectroscopy, and the conductivity of cytochrome c_3 film under higher hydrogen pressures of $3 \sim 5$ MPa.

EXPERIMENTAL

Sample of cytochrome c (horse heart, c-7752, molecular weight = 12384) was purchased from Sigma Chemical Co. The following buffer solutions were used: phthalic acid buffer (pH = 4.01), phosphoric acid buffer (pH = 6.86), boric acid buffer (pH = 9.18), and tris buffer (pH = 7–9). In this pH range, it is know that the denaturation does not occur. After removal of dissolved air, these solutions were exposed to the hydrogen gas of $1.3 \sim 1.5$ atm, and UV-visible spectra were measured.

The film of oxidized cytochrome c was prepared from water solution of cytochrome c in quartz cell by use of film-casting method. This cast film was exposed to the hydrogen of $1.5 \sim 3.0$ atm, and UV-visible-near infrared spectra were measured.

Purified cytochrome c_3 and hydrogenase were obtained from sulfate-reducing bacteria D. vulgaris, strain Miyazaki. The cytochrome c_3 preparation was dialyzed thoroughly against distilled water.

The thin film of cytochrome c_3 is prepared on a quartz plate with Au electrode from aqueous solution of cytochrome c_3 and a bit of hydrogenase (reductase) by cast method [2]. The ratio of hydrogenase to cytochrome c_3 is about 1 to 1000 in the number of molecule. The surface type sample cell is set into a sealed measurement chamber. Then, cytochrome c_3 film is dried by the vacuum system with a cold trap. The thickness of film is evaluated to about 70 nm. Finally, hydrogen gas is introduced in the pressure range of $2 \sim 5$ MPa. The conductivity is measured during and after the reduction of cytochrome c_3.

RESULTS AND DISCUSSION

Self-reduction

Figure 1 shows UV-visible spectra of cytochrome c water solution under the hydrogen pressure of 1.5 atm. The isosbestic points appeared at 557, 542, 526, 506, 432, 409, and 337 nm. The rate constant of the apparent pseudo first-order equilibration reaction evaluated by the fitting was $4.5 \times 10^{-6}\,s^{-1}$.

The film of oxidized cytochrome c was prepared by film-casting method and UV-visible spectra were measured under the hydrogen gas of 1.5 or 3.0 atm. The splitting of the alpha peak and the beta peak with increased absorbance indicates that the self-reduction occurred.

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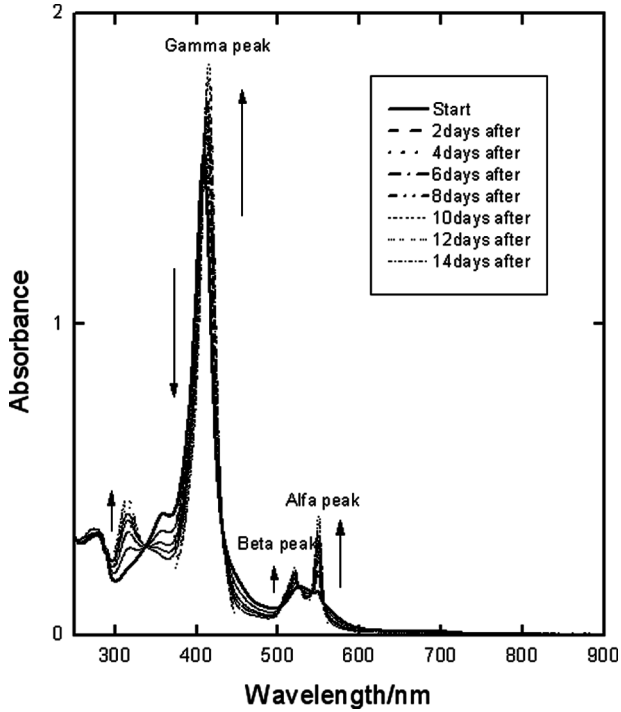

FIGURE 1 UV-visible spectra of cytochrome c water solution from 900 nm to 250 nm.

In the equilibrium, the molar fractions of reduced form are about 0.16 at 3.0 atm and about 0.12 at 1.5 atm.

As to the buffer effect, at pH 4, the spectrum of cytochrome c did not change under the same temperature and hydrogen pressure as the experiment in Figure 1. In the water solution systems at pH 7, while the distilled water solution system shows the change from the oxidized form to the reduced form, the buffer solutions of both the phosphoric acid buffer (pH = 6.86) and tris buffer showed the small reduced rate and the reduced ratio such as 0.1–0.2. In the water solution systems at pH 9, the buffer solution of both the boric acid buffer (pH = 9.18) and tris buffer also the same behavior. The same effect appears by adding NaCl to the distilled water solution of cytochrome c at the concentration of 0.001 to 0.01 M.

These facts suggest that the environments of heme play the important role in the redox mechanisms and such environments change with pH and the sort/concentration of salts.

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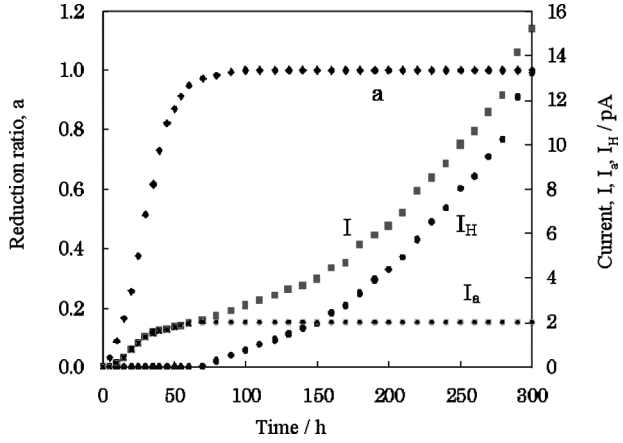

FIGURE 2 The time course of the reduction ratio and the current of cytochrome c_3 film.

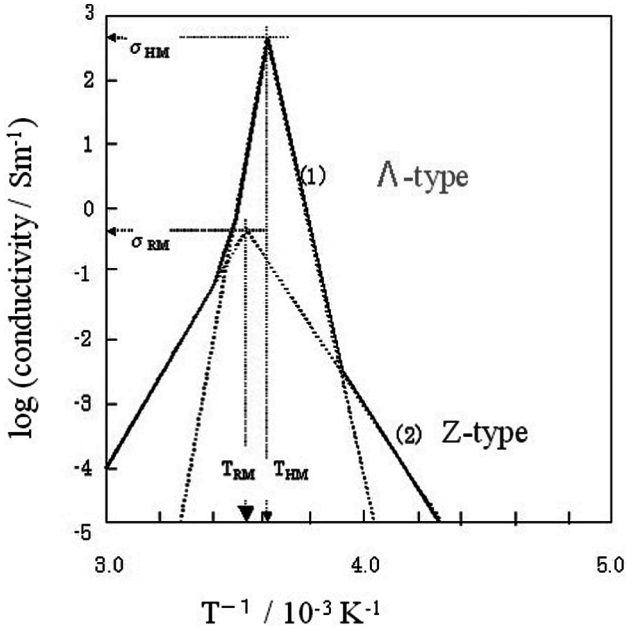

FIGURE 3 The temperature dependence of dc conductivity for ferrocytochrome c_3 film under the high pressure range of hydrogen gas, 2950 kPa. (1) This component corresponds to I_R due to the effect of the heme-reduction. (2) This component corresponds to I_H due to the effect of hydrogen ions (H^+) under the high pressure range of hydrogen gas.

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Conductivity

Ferri-cytochrome c_3 film with a bit of hydrogenase changes gradually to ferro-cytochrome c_3 film after hydrogen gas is introduced in the chamber. The plots drawn in Figure 2-(a) present the time-dependence of the heme reduction-ratio "a(t)" of cytochrome c_3 film at 305 K under hydrogen-gas pressure, 2 MPa. a(t) reaches the saturation-value "1" after 1.9×10^5 s.

Even after the reduction, the current increase over 300 hours as shown in Figure 2-(I). After 2600 hours, the current value reaches to 5.2×10^{-5} A with the applied voltage of 0.1 V. This value is correspondent to 1.3×10^2 S/m in conductivity.

The current "I" in Figure 2 seems to be constructed from two components, I_R and I_H. The "I_R" has a close relation to the reduction-ratio "a(t)". The extrapolated saturation value is 2.0×10^{-12} A. This conductivity value is about 6.2×10^{-8} S/m (σ_{RS}) at 305 K. The I_H-component continues to increase slowly over very long time ever after the hemes were reduced. It approaches to the saturation value 5.2×10^{-5} A after 2600 hours; I_H equals I.

Under hydrogen pressure of 3.0 MPa, I_H has the value of around 10^3 times of I_R. When $I_H > I_R$, an inverse V-form component ($\wedge$-type) of the

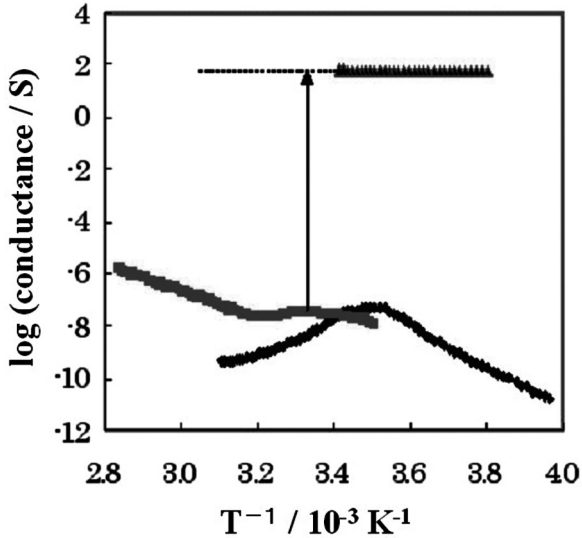

FIGURE 4 Transition from semiconductive state to metallic state for ferro-cytochrme c_3 film. (See COLOR PLATE V)

conductivity is added to Z-type, as shown in Figure 3. The maximum value (σ_{HM}) of σ_H is 1.3×10^2 S/m at 286 K (T_{HM}) under the hydrogen pressure of 3.0 MPa. The activation energy is 2.7 eV in the range of $T < 286$ K and is 1.6 eV in the range of $T > 286$ K.

Under the hydrogen pressure of more than around 3 MPa, the conductivity jumps to be higher as shown in Figure 4. The conductivity value is around 10^6 S/m at 250–330 K. The coefficient of temperature in the metallic state is $0.0015 \sim 0.0025\,\Omega K^{-1}$. These values are approximately same as compared with those of metals. This transition is considered to be due to the doping of electron and change in the electronic state of cytochrome c_3 molecules.

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