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ELECTROCATALYTIC FOUR-ELECTRON REDUCTION OF OXYGEN AT THE CYTOCHROME c_3-ADSORBED ELECTRODE

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The electrocatalytic activity of cytochrome c_3 for the reduction of molecular oxygen was characterized from the studies of the adsorption of cytochrome c_3 and the co-adsorption of cytochrome c_3 with cytochrome c on the mercury electrode by the a.c. polarographic technique. The adsorption of cytochrome c_3 on the mercury electrode is irreversible and is diffusion-controlled. The maximum amount of cytochrome c_3 adsorbed was $0.92 \cdot 10^{-11}$ mol $\cdot$ cm^{-2} at -0.90 V. The amount of cytochrome c_3 in the mixed adsorbed layer with cytochrome c was determined from the differential capacitance measurement. It was shown that the fractional coverage of cytochrome c_3 can be estimated from its bulk concentration and the diffusion coefficient ($1.05 \cdot 10^{-6}$ cm$^2 \cdot$ s^{-1}). Cytochrome c_3 catalyzes the electrochemical reduction of molecular oxygen from the two-electron pathways via hydrogen peroxide to the four-electron pathway at the mercury electrode in neutral phosphate buffer solution. The catalytic activity varies with the bulk concentration of cytochrome c_3. The highest catalytic activity for the oxygen reduction (no hydrogen peroxide formation) is attained when one-half of the mercury electrode surface is covered by cytochrome c_3. The addition of cytochrome c or bovine serum albumin to the cytochrome c_3 solution inhibits the catalytic activity of cytochrome c_3. The reversible polarographic behavior of cytochrome c_3 through the mixed adsorbed layer of cytochrome c_3 and cytochrome c was also investigated.

Studies on electrocatalysis of oxygen reduction would have a great significance in the development of the energy conversion system. Brdicka and Tropp [1] found that heme proteins catalyzed the polarographic reduction of oxygen. Swedin [2] extended their investigation further by using various heme proteins. A number of papers have been published on the electrocatalytic reduction of oxygen in the presence of various phthalocyanines and porphyrins since 1964. The electrocatalytic reduction of oxygen by biological substances such as cytochrome c, hemoglobin and hemin was also reported [3]. The addition of chlorin e_6 and hemin to solutions of pH 12.1 causes the change of the reaction scheme for the reduction of molecular oxygen on an amalgamated gold electrode from two-electrode paths to the direct four-electron path [3]. Scheller and coworkers [4] studied the activity of cytochrome *P*-450 in the polarographic reduction of oxygen. At the electrode surface covered by cytochrome *P*-450 the reaction of proton with HO_2^-, which is the reduction intermediate of oxygen, is hindered and the complex formation of HO_2^- with ferricytochrome *P*-450 may be favored. This interaction causes both the shift of the half-wave potential of the second wave to the positive direction and the increase of the first-wave height. Tarasevich and coworkers [5] investigated the electrochemical reduction of molecular oxygen at carbon black electrode on which laccase was immobilized in oxygen-saturated acetate-phosphate buffer solution at pH 5.5. With the introduction of laccase, the electrode potential shifts about 500 mV towards the positive direction

and molecular oxygen is reduced directly to water.

Molecular oxygen is also reduced directly to water in alkaline solutions at carbon electrodes, in which monomeric manganese phthalocyanine, polymeric manganese phthalocyanine, polymeric cobalt phthalocyanine, or cobalt tetraphenylporphyrin is impregnated [6]. Collman et al. [7] found in the electrochemical reduction of oxygen that the pyrolytic graphite disk with immobilized dimeric face-to-face cobalt porphyrin shows a high catalytic activity towards the reduction of molecular oxygen to water with no significant hydrogen peroxide produced.

The reduction of oxygen to hydrogen peroxide in acidic and neutral solutions is catalyzed by phthalocyanines, porphyrins, and biological substances such as cytochrome *c*, hemoglobin and hemin [3,6].

The present work deals with the adsorption of cytochrome c_3, the simultaneous adsorption of cytochrome c_3 with cytochrome *c* on a mercury electrode, and the electrocatalytic reduction of molecular oxygen by cytochrome c_3 at a dropping mecurcy electrode in neutral phosphate buffer solution.

The physicochemical properties of cytochrome c_3 purified from *Desulfovibrio vulgaris*, strain Miyazaki, have been studied extensively in our laboratory [8]. It is similar to mammalian cytochrome *c* in molecular weight and in absorption spectrum, but contains four hemes in the molecule. The electrode reaction of cytochrome c_3 is reversible and diffusion-controlled [9]. The electrochemical reduction from the ferri form to the ferro form is considered to be four one-electron steps and hemes in the molecule are non-equivalent. The apparent formal potential of the ferri-ferrocytochrome c_3 couple is −0.528 V vs. saturated calomel electrode (−0.287 V vs. normal hydrogen electrode) [10]. The redox potential of hemes in the molecule is estimated to be $E_1 = -0.467$, $E_2 = -0.519$, $E_3 = -0.539$ and $E_4 = -0.580$ V vs. saturated calomel electrode from the computer simulation of the differential pulse polarogram [11].

Materials and Methods

Materials

The cultivation of *D. vulgaris*, strain Miyazaki, and the extraction of cytochrome c_3 from bacterial cells were given elsewhere [8]. The purity index of cytochrome c_3 was 3.0 and the purity was considered to be nearly 100%. The concentration of cytochrome c_3 was measured by a spectrophotometric method with a Hitachi Model 124 spectrophotometer. Horse heart cytochrome *c* was the product of Boehringer Mannheim GmbH and its concentration was also measured by a spectrophotometric method. Bovine serum albumin was obtained from Sigma Chemical Co. Other chemicals were of analytical reagent grade.

Procedures.

Pulse polarographic measurements were made with a Fuso Polarograph Model 312 and phase sensitive a.c. polarographic measurements were made with a Fuso Potentiostat Model 311 and a Fuso Phase Sensitive Detector Model 332 (Fuso and Co., Kawasaki, Japan).

A three-compartment water-jacketed electrochemical cell was used and each compartment was separated by a porous Vycor glass so that the contamination of oxygen could be minimized. The working electrode was a dropping mercury electrode and the flow rate of mercury was 1.315 $mg \cdot s^{-1}$ at open circuit in 0.03 M phosphate buffer solution at pH 7.0. The counter electrode was a mercury pool in the working electrode compartment. The reference electrode was a saturated calomel electrode which was separated from the working electrode compartment by a porous Vycor glass. The reference electrode was coupled with a platinum wire (0.3 mm diameter) electrode in the working electrode compartment through a 0.24 μF capacitor to improve the response at higher frequencies in a.c. polarographic measurements [12, 13].

Cytochrome c_3 in 0.03 M phosphate buffer solution was transferred to the electrochemical cell (2 ml), and then it was deaerated for 15–20 min by bubbling with purified argon. Argon gas was purified by bubbling through an acidic vanadous solution and then it was washed by distilled water. The same procedure was also applied to the mixed solutions of cytochrome c_3 and cytochrome *c*, and those of cytochrome c_3 and bovine serum albumin. The electrochemical reduction of oxygen was studied in air-saturated solutions at 1 atm at 25°C.

All the experiments were carried out at 25°C and the electrode potentials were referred to the saturated calomel electrode.

Results

Adsorption of cytochrome c_3 on mercury electrode. The differential capacitance-time curves of the dropping mercury electrode in heme protein solutions (cytochrome c_3 and cytochrome c) revealed that the adsorption of these heme proteins on the mercury electrode is diffusion-controlled [14,15]. That is, the amount of the adsorbed heme protein can be evaluated by using Koryta's equation [16]. The surface coverage, $\theta = \Gamma/\Gamma_{max}$, was evaluated from the concentration dependence of the differential capacitance at the dropping mercury electrode [17], where Γ is the surface concentration of the heme protein and Γ_{max} the maximum surface concentration. At concentration higher than $1.03 \cdot 10^{-5}$ M, the dropping mercury electrode (drop time 4 s) showed a saturation value which corresponded to the complete coverage of the mercury drop surface as shown in Fig. 1. The maximum surface concentration, Γ_{max}, was calculated from the concentration at $\theta = 1$ by using Koryta's equation [16]. The maximum concentration obtained was $0.92 \cdot 10^{-11}$ mol $\cdot$ cm^{-2} at -0.90 V.

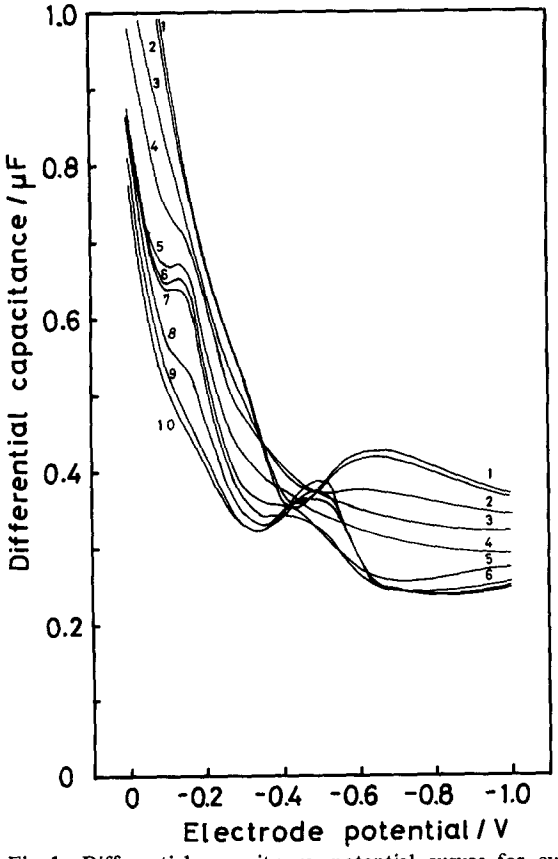

Fig. 1. Differential capacity vs. potential curves for cytochrome c_3 at different concentrations in 0.03 M phosphate buffer solution at pH 7.0. Drop time, 4 s; surface area of the electrode, $2.53 \cdot 10^{-2}$ cm^2; concentration of cytochrome c_3 (M) (1) 0; (2) $1.06 \cdot 10^{-6}$; (3) $1.59 \cdot 10^{-6}$; (4) $2.65 \cdot 10^{-6}$; (5) $3.71 \cdot 10^{-6}$; (6) $5.30 \cdot 10^{-6}$; (7) $1.03 \cdot 10^{-5}$; (8) $3.61 \cdot 10^{-5}$; (9) $7.21 \cdot 10^{-5}$; (10) $1.03 \cdot 10^{-4}$.

On the other hand, the maximum surface concentration of cytochrome c on mercury electrode was reported to be $0.76 \cdot 10^{-11}$ mol $\cdot$ cm^{-2} in 0.1 M KCl [18] and $0.65 \cdot 10^{-11}$ mol $\cdot$ cm^{-2} 0.1 M ammonia buffer solution [19].

a.c. polarographic behavior of cytochrome c_3. a.c. polarographic behavior of cytochrome c_3 was peculiar. At lower frequencies, both resistance and capacitance peaks were observed at the half-wave potential of pulse polarography. With the increase of the a.c. frequency, the resistance peak became smaller and smaller and finally it disappeared at the frequencies above 2000 Hz as shown in Fig. 2. That is, the resistive component due to the mass transport of cytochrome c_3 from the bulk of the solution to the electrode surface is considered to be negligible at higher frequencies and the charge transfer resistance of the adsorbed cytochrome c_3 on the mercury electrode is also negligible. The capacitive peak height was frequency independent, when the electrode surface was fully covered. From these experimental results, it is reasonable to assume that the electrode reaction of cytochrome c_3 at the mercury electrode is well-represented by the Laitinen-Randles equivalent circuit [20].

The maximum surface concentration of cytochrome c_3 can be calculated by using the equation for the Laitinen-Randles equivalent circuit:

$$C_a = nn_{app}F^2x(1-x)\Gamma/RT \qquad (1)$$

where C_a is the differential capacitance due to the adsorbed cytochrome c_3, n the number of electrons involved in the electrode reaction, n_{app} the apparent number of electrons calculated from the Nernst slope of the potentiometric measurements, x the fraction of ferricytochrome c_3 in the adsorbed layer, Γ the surface concentration, and R, T, and F have their usual significance. Under the conditions that the con-

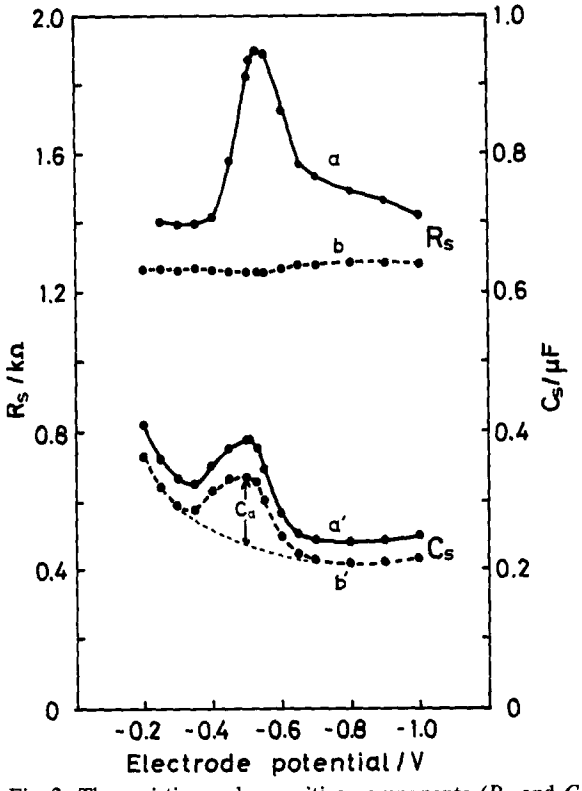

Fig. 2. The resistive and capacitive components (R_S and C_S are in series) of dropping mercury electrode in $1.03 \cdot 10^{-4}$ M cytochrome c_3 (supporting electrolyte is 0.03 M phosphate buffer at pH 7.0) at (a, a') 100 Hz and (b, b') 2000 Hz. Drop time 4 s; surface area of the electrode, $2.35 \cdot 10^{-2}$ cm^2.

ditions that the concentration of cytochrome c_3 is higher than $1.03 \cdot 10^{-5}$ M and that the dropping time of the dropping mercury electrode is 4 s, the surface concentration of cytochrome c_3 on mercury electrode is attained to its maximum value in the potential range studied. The apparent number of electrons involved in the electrode reaction is 0.64 [10]. The fraction of ferricytochrome c_3 in the absorbed layer and C_a at the half-wave potential are 0.5 and 3.83 μF cm^{-2} respectively, and the maximum surface concentration of cytochrome c_3 determined in the preceding section is $0.92 \cdot 10^{-11}$ mol $\cdot$ cm^{-2}. These values lead to the number of electrons involved in the electrode reaction of the adsorbed cytochrome c_3 to be 0.75 instead of 4.0. The capacitance-potential profile for the adsorbed cytochrome c_3 calculated from Eqn. 1 fits reasonably well with the experimental curve shown in Fig. 2.

The electron transfer mechanism between the electrode and the adsorbed cytochrome c_3 is being studied. However, the amount of the adsorbed cytochrome c_3 on the mercury electrode can be estimated from the value of C_a.

Simultaneous adsorption of cytochrome c_3 and cytochrome c on the mercury electrode. It is usually difficult to determine the surface concentration of each protein in a mixed adsorbed layer on the mercury electrode. However, in the case of the simultaneous adsorption of cytochrome c_3 with an electrochemically inactive protein, e.g. cytochrome c, it is possible to evaluate the amount of cytochrome c_3 in the adsorbed layer on the mercury electrode, provided that the Laitinen-Randles equivalent circuit model is applicable. The ratio of the capacitance peak due to the adsorbed cytochrome c_3 in $5.6 \cdot 10^{-5}$ M cytochrome c_3 solution to that in the mixed solution of $5.6 \cdot 10^{-5}$ M cytochrome c_3 and $5.6 \cdot 10^{-5}$ M cytochrome $c3$ in the adsorbed layer is estimated to be 0.41.

If we assume that the adsorptions of both cytochrome c_3 and cytochrome c are irreversible and diffusion-controlled, and that there is no interaction between these proteins, the amount of each protein on the mercury electrode can be determined by using Koryta's equation. The diffusion coefficients of cytochrome c_3 and cytochrome c are $1.05 \cdot 10^{-6}$ cm$^2 \cdot$ s^{-1} and $0.95 \cdot 10^{-6}$ cm$^2 \cdot$ s^{-1} [21–23], respectively, and the areas occupied by the adsorbed protein on the mercury electrode at the saturation coverage are 1800 Å^2 and 2200 Å^2 [15], respectively. The fraction of cytochrome c_3 in the adsorbed layer is estimated to be 0.46 which agrees well with the value determined from the differential capacitance peak due to the adsorbed cytochrome c_3.

Electrocatalytic reduction of oxygen by cytochrome c_3. Typical polarograms for the reduction of oxygen in 0.03 M phosphate buffer solution at pH 7.0 with and without ferricytochrome c_3 are shown in Fig. 3, in which the correction was made for the reduction wave due to cytochrome c_3. The half-wave potentials of the first wave for the reduction of oxygen in the presence and in the absence of ferricytochrome c_3 were practically the same, but the first wave height was increased by the addition of ferricytochrome c_3. Finally, the second wave merged into the first wave in the solution of about $3 \cdot 10^{-6}$ M

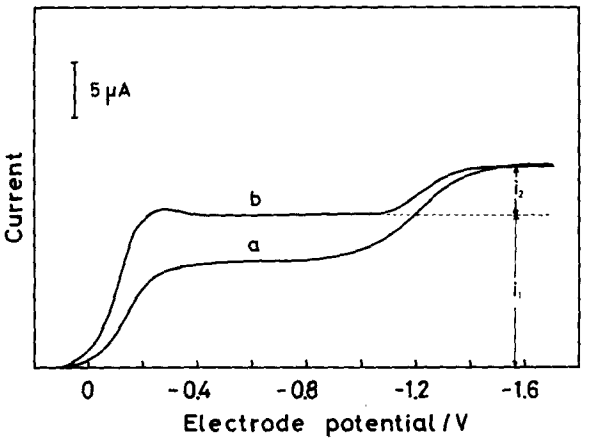

Fig. 3. Pulse polarogram of the reduction of oxygen in air-saturated 0.03 M phosphate buffer solution at pH 7.0 (a) in the absence and (b) presence of $5.6 \cdot 10^{-5}$ M cytochrome c_3. Drop time 2 s; sampling time 50 ms; surface area of the electrode, $1.62 \cdot 10^{-2}$ cm^2.

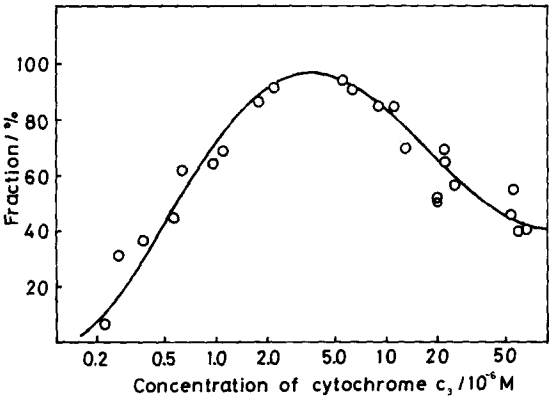

Fig. 4. Fraction of the four-electron reduction of oxygen on dropping mercury electrode at different concentrations of cytochrome c_3 in 0.03 M phosphate buffer solution at pH 7.0. Conditions were the same as in Fig. 3.

ferricytochrome c_3. With a further increase in the concentration of ferricytochrome c_3, the second wave for the reduction of hydrogen peroxide appeard again and its wave height increased gradually and reached a constant value. The half-wave potential of the second wave, which corresponds to the reduction of hydrogen peroxide to water, was not affected by the addition of ferricytochrome c_3. The ratio of the first wave height to the second wave height varied with the amount of ferricytochrome c_3 added. However, the sum of the first wave height, i_1, and the second wave height, i_2, remained the same regardless of the presence or the absence of ferricytochrome c_3.

In the potential region of the first wave, it is plausible to assume that the co-reduction of oxygen via two-electron and four-electron pathways takes place. The fraction of the four-electron path – the direct reduction of oxygen to water – in the limiting current region of the first wave is given by $(i_1 - i_2)/(i_1 + i_2)$ and that of the two electron-path is $2i_2/(i_1 + i_2)$. The fraction of the direct four-electron reduction of oxygen to water with respect to the ferricytochrome c_3 concentration is shown in Fig. 4. The maximum value, in which nearly 100% of oxygen is reduced via the four-electron path, is attained at the surface coverage of 40–50% ($3 \cdot 10^{-6}$–$4 \cdot 10^{-6}$ M cytochrome c_3 solution). When the mercury electrode is fully covered by cytochrome c_3, the fraction of the four-electron path decreases down to 40–50%.

Effect of cytochrome c on the four-electron reduction of oxygen catalyzed by cytochrome c_3. Brdicka and Tropp [1] and Betso et al. [24] have noticed that cytochrome *c* catalyzed the electrochemical reduction of oxygen on mercury electrode directly to water. The investigation by Tarasevich and Bogdanovskaya [3] indicated that the half-wave potential of the first wave of oxygen reduction shifted by about 100 mV towards the positive direction in a neutral solution of cytochrome *c* and that the slight increase in the limiting current of the first wave was observed. In the present investigation, however, we noticed that the addition of cytochrome *c* and bovine serum albumin cuased no observable change in the polarographic reduction wave of oxygen.

In order to elucidate the electrochemical reduction mechanism of oxygen in the presence of cytochrome c_3, the effect of electrocatalytically inactive cytochrome *c* and bovine serum albumin on the electrode reaction of molecular oxygen was studied in $4 \cdot 10^{-6}$ M cytochrome c_3 solution, in which nearly 100% of oxygen is reduced directly to water. The addition of cytochrome *c* caused the decrease in the fraction of the four-electron reduction of oxygen as shown in Table I. The fraction of the surface coverage by cytochrome c_3 and cytochrome *c* calculated by using Koryta's equation is also shown in Table I. In the mixed solutions of $5.6 \cdot 10^{-5}$ M cytochrome c_3 and $5.6 \cdot 10^{-5}$ M cytochrome *c*, and $5.6 \cdot 10^{-5}$ M

TABLE I

EFFECT OF CYTOCHROME *c* CONCENTRATION ON THE FRACTION OF TE FOUR-ELECTRON REDUCTION OF OXYGEN

Concentration of cytochromes (M)		Surface coverage (θ)		Fraction of four-electron reduction
Cytochrome c_3	Cytochrome *c*	Cytochrome c_3	Cytochrome *c*	
$4 \cdot 10^{-6}$	–	0.46	–	1.00
$4 \cdot 10^{-6}$	$2 \cdot 10^{-6}$	0.46	0.27	0.78
$4 \cdot 10^{-6}$	$4 \cdot 10^{-6}$	0.46	0.54	0.45
$4 \cdot 10^{-6}$	$8 \cdot 10^{-6}$	0.15	0.85	0.28
$4 \cdot 10^{-6}$	$2 \cdot 10^{-5}$	0.07	0.93	0.18

cytochrome c_3 and $5.6 \cdot 10^{-5}$ M bovine serum albumin, the fraction of the four-electron reduction of oxygen was negligible. The polarograms corrected for the reduction of the base solutions were identical to those obtained in the air-saturated buffer solution.

Discussion

In order to avoid the release of radicals such as $OH^{\cdot}$ and $H^{\cdot}$ and moieties such as H_2O_2 in the course of the reduction of oxygen in biological systems, the electrons must be released cooperatively to reduce oxygen directly to water. A set of single electron transfer centers is common to many complex redox reactions in multienzyme systems, such as cytochrome oxidase (2 heme, 2 Cu), cytochrome c_3 (4 heme), laccase (4 Cu), ascorbate oxidase (8 Cu), and L-tryptophan oxygenase (2 heme, 2 Cu). On the other hand, in the cases of the catalytic reduction of oxygen by many monometallic macrocyclic complexes, the two-electron reduction to hydrogen peroxide is always dominant, probably because these catalysts have a single metal center.

Collman and coworkers [7] synthesized a 'face-to-face' cobalt porphyrin as a catalyst for the reduction of oxygen directly to water. They speculated that a binuclear 'face-to-face' cobalt macrocyclic complex strongly binds dioxygen between the porphyrin rings. The fraction of the four-electron reaction path is strongly influenced by the distance between the two cobalt atoms.

In the case of cytochrome c_3 from *D. desulfuricans*, strain Norway, however, the compact organization of the four hemes within the molecule is reported [25]. These hemes lies near the surface of the protein and the iron-to-iron distances range from 10.9 to 17.3 Å, which are much greater than the cavity of binuclear 'face-to-face' porphyrin. The structural arrangement of cytochrome c_3 from *D. vulgaris* is considered to be similar to that from *D. desulfuricans*. The present measurements on the surface concentration of cytochrome c_3 from *D. vulgaris* on mercury electrode indicated that the extent of the deformation (or unfolding) of cytochrome c_3 is considered to be small. The cooperation of hemes with oxygen in the molecule is improbably because of the compact arrangement of the molecule. It is obvious from this experimental evidence that the reaction scheme proposed by Collman et al. [7] for the four-electron reduction of oxygen is not applicable to the catalytic reduction of oxygen by cytochrome c_3. It is plausible to assume that oxygen is activated in the cavities between cytochrome c_3 molecules at the electrode surface because the highest fraction of the four-electron reduction is attained when half of the mercury electrode surface is covered by cytochrome c_3 as shown in Fig. 4. This assumption is also evidenced from the results that the addition of electrocatalytically inactive proteins, which reduce the cavities for activating oxygen on the electrode, markedly suppresses the fraction of the four-electron reduction of oxygen as shown in Table I. On the fully covered electrode surface by cytochrome c_3, the enhancement of the first oxygen wave can still be observed. On the other hand, in the mixed solution of $5.6 \cdot 10^{-5}$ M cytochrome c_3 and $5.6 \cdot 10^{-5}$ M cytochrome *c*, the

TABLE II

EFFECT OF CYTOCHROME *c* CONCENTRATION ON THE LIMITING CURRENT OF THE REDUCTION OF FERRICYTOCHROME c_3

Concentration of cytochromes (M)		Surface coverage (θ)		i_{obs}/i_{calc}
Cytochrome c_3	Cytochrome *c*	Cytochrome c_3	Cytochrome *c*	
$1.03 \cdot 10^{-4}$	–	1.00	–	1.00
$0.84 \cdot 10^{-4}$	$0.28 \cdot 10^{-4}$	0.72	0.28	0.93
$0.56 \cdot 10^{-4}$	$0.56 \cdot 10^{-4}$	0.46	0.54	0.63
$0.28 \cdot 10^{-4}$	$0.84 \cdot 10^{-4}$	0.22	0.78	0.35

four-electron reduction of oxygen is completely inhibited, even though the fraction of cytochrome c_3 in the adsorbed layer on the dropping mercury electrode is estimated to be 0.41. This is probably due to the formation of the dense adsorbed layer of cytochrome *c*, which deactivated the active center of the oxygen reduction.

The limiting current for the reduction of cytochrome c_3 was suppressed by the addition of cytochrome *c*. However, both the half-wave potential of pulse polarogram and the peak potential of differential pulse polarogram were not altered. The relation between the fraction of cytochrome c_3 in the fully covered electrode surface by both cytochrome c_3 and and cytochrome *c* and the degree of suppression of the limiting current for the reduction of ferricytochrome c_3 (in arbitrary units) is shown in Table II. This result indicates that only the sites occupied by cytochrome c_3 act as the active centers for the electron transfer between the electrode and ferricytochrome c_3 from the bulk of the solution. On the other hand, the electron transfer mechanism to oxygen molecule was not influenced by the adsorbed layer of cytochrome *c* or bovine serum albumin on mercury electrode.

The polarographic behavior of oxygen in the presence of cytochrome c_3 may be similar to that with cytochrome *P*-450 reported by Scheller et al. [4]. That is, ferricytochrome c_3 forms a complex with peroxide, a reduction intermediate of oxygen, and it is further reduced to water at potentials near the half-wave potential of the first oxygen wave. In the mixed adsorbed layer of cytochrome c_3 and cytochrome *c*, the number of adjacent adsorbed cytochrome c_3 molecules, which act as an active center for the four-electron reduction of oxygen, is probably quite small and the enhancement of the first oxygen wave is not observable. The mechanism of the four-electron reduction of oxygen by macrocyclic complex polymers [6] is probably similar to that by cytochrome c_3 because the active center of both molecules is exposed to the solution.

The other possible mechanism of the four-electron reduction of oxygen is a catalytic decomposition of hydrogen peroxide which is formed on the first reduction wave as in the case of the reduction of oxygen in the presence of molybdate ion [26]. The half-wave potential of the reduction of oxygen to hydrogen peroxide is close to that of the four-electron reduction of oxygen and the reaction is kinetically-controlled. We examined the temperature dependence of the fraction of the four-electron reduction of oxygen in the temperature range 15–35°C in $2 \cdot 10^{-5}$ M cytochrome c_3 solution and the fraction was found to be almost independent of temperature. This result indicates that the disproportionation reaction mechanism is unlikely to explain the temperature-independent catalytic reduction of oxygen in cytochrome c_3 solution.

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Note added in proof (Received May 6th, 1981)

The structure of cytochrome c_3 from *D. vulgaris*, strain Miyazaki, has been determined by Higuchi et

al. [27] at 2.5 Å resolution by X-ray diffraction and the overall shape, especially the relative orientations of the four hemes, resembles that of *Desulforibrio vulgaris* Norway.

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