Spectroelectrochemical Determination of Redox Potential of Cytochrome *c'* in *Rhodospirillum rubrum* Chromatophores

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Synopsis. A spectroelectrochemical technique has been used for redox study of cytochrome *c'* in chromatophores in the presence of Toluidine Blue O as a mediator. The midpoint potential of cytochrome *c'* thus determined was 0 mV (n=1) at pH 7.0, which agreed well with the value determined by redox titration.

Studies of the redox properties of the components in photosynthetic and other several biological electron-transport chains become more important for understanding of their reaction mechanism, combined with the development of new disintegrating methods of biomembrane. Although the thin-layer spectroelectrochemistry[1] offers several advantages for the study of the redox chemistry of biological electron-transport components, many difficulties to be solved lies under our eyes. One of the difficulties is of measuring small absorbance change between oxidized and reduced forms of electroactive species due to the very short light path length in a thin-layer cell and extremely dilute suspension of the disintegrated membrane. So, few spectroelectrochemical studies on redox components in biomembrane have been reported so far except for study on fluorescence yield of chloroplast fragments,[2] whereas many studies on electron-transport components in soluble state[3-6] can be found. But this problem can be overcome to some extent by integrating the spectrum data with use of a microcomputer. This paper describes an attempt of such spectroelectrochemical measurement of indirect reduction of cytochrome *c'* in chromatophore membrane from *Rhodospirillum rubrum* (G-9).

Experimental

Chromatophores were prepared from the light grown cells of *Rs. rubrum* (G-9) according to the method described previously.[7] Chromatophores were suspended in 0.6 M (M=mol dm^{-3}) GTA buffer, consisting of a mixture of 0.2 M 3,3-dimethylglutaric acid, 0.2 M tris(hydroxymethyl)aminomethane and 0.2 M 2-amino-2-methyl-1,3-propanediol, which was adjusted to various pH by adding HCl or NaOH, and the concentration of chromatophores was expressed in terms of absorbance of bound bacteriochlorophyll at 873 nm (A_{873}). The sample solution was deoxygenated prior to use by vacuum-nitrogen cycling on a vacuum-purified nitrogen double manifold and loaded into a thin-layer cell by using rubber septum caps and syringe techniques,[6] because of the autooxidizability of cytochrome *c'*. The spectroelectrochemical thin-layer cell was used in a Hitachi Model 557 spectrophotometer equipped with Hitachi MB-6890 microcomputer at 25 °C. Our present spectroelectrochemical thin-layer cell design was reported previously.[8] The midpoint potential (E_m) was determined by sequentially applying a series of potentials (E_{appl}) against a micro Ag/AgCl electrode using a Hokuto Model HA-501 potentiostat. Each potential was maintained until electrolysis ceased so that the equilibrium of the ratio of the concentration of oxidized to reduced from ([Ox]/[Red]) was established as defined by the Nernst equation (for about 10—60 min), and each corresponding value of [Ox]/[Red] to the series of E_{appl}'s was estimated from the absorbance change at 430 nm. The absorbance spectra were recorded as the average of five times scanning for improvement the ratio of signal to noise.

Results and Discussion

It was suggested by a conventional electrochemical method that cytochrome *c'* in chromatophores could not directly exchange electrons at an electrode, presumably due to the insulation of redox site from the electrode by surrounding proteins. However, spectroelectrochemical measurement can be made on cytochrome *c'* in chromatophores by adding another redox species (a mediator-titrant[9]), which transports electrons between the electrode and cytochrome *c'*, to the suspension. The ideal properties for the mediator were discussed in detail.[9] Among the redox reagents tested, Toluidine Blue O (E_m=+34 mV at pH 7.0[10]) was finally chosen as the mediator, fulfilling some properties for mediator, and the properties were examined in some detail by means of conventional electrochemical and spectrophotometric techniques as described.[11] So, all the measurements described hereafter were carried out in the presence of 50 μM Toluidine Blue O and the concentration of cytochrome *c'* in chromatophores was adjusted to 4.2 μM (estimated from A_{873}=100[12]).

The computer-calculated reduced-minus-oxidized difference absorption spectra at several steps of electrolytic reduction are shown in Fig. 1.

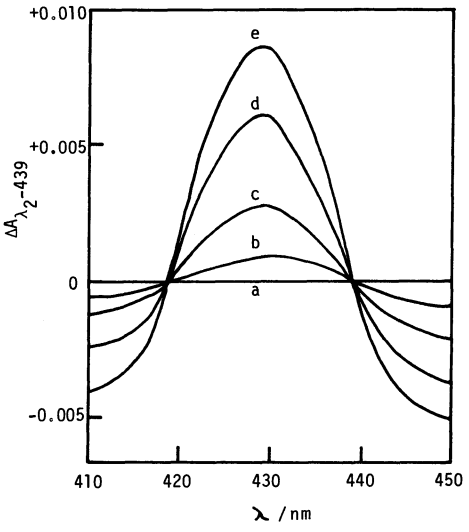

Fig. 1. Difference absorption spectra of cytochrome *c'* in chromatophores at thin-layer cell for series of applied potentials at pH 7.0.
E_{appl}; (a): +75, (b): +25, (c): 0, (d): −25, (e): −75 mV.

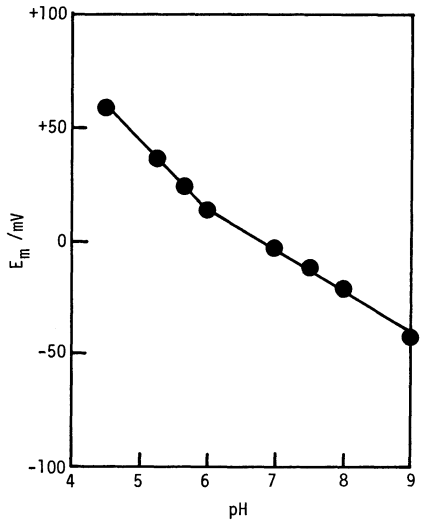

Fig. 2. Effect of pH on E_m values of cytochrome c' in chromatophores.

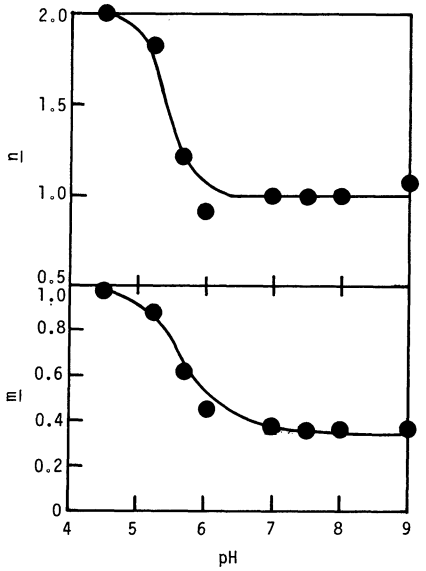

Fig. 3. Values of n and m at various pH's for cytochrome c' reduction.

The Nernst plots at various pH's showed good linear relationships. The E_m at pH 7.0 was 0 mV, which agreed well with the value determined by redox titration.[13] The pH-dependence of E_m was divided in two straight lines; one with a slope of −29 mV/pH in the acidic region below pH 6.0, and the other with −21 mV/pH in the more basic region. This result indicates the presence of a heme-linked ionization probably in the reduced form as described by Clark.[14] The ionization constant (K_{red}) became to be 1×10^{-6} M (pK_{red}=6.0). This result is supported by a spectrophotometric result on pH-dependence of absorbance at 430 nm for electro-reduced cytochrome c', where pK_{red} was estimated to be 5.95 according to the method by Moore et al.[15]

The number of electrons (n) involved in the reduction process was 2 in more acidic region than pH 5.0 and 1 in more basic region than pH 6.0 (Fig. 3). Values for the number of protons transferred (m) accompanying with cytochrome c' reduction are also shown in Fig. 3 as a function of pH. The value of m was about 1 near pH 5.0, and 0.4 in the more basic region. The small value of m may indicate the conformational change of cytochrome molecule or chromatophore membrane with pH change and/or the considerably slower rate of proton transfer than that of electron transfer probably due to hydrophobicity of the surrounding proteins.

In conclusion, it became clear that this spectroelectrochemical technique was very useful to determine the redox potential of the extremely small amounts of redox components in a biomembrane or the fragments such as photosynthetic reaction center, bc_1 and b_6f complexes.

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