CAPSULES notes and applications from BASi

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April 2000

Measurement of the Redox Potential of Outer Mitochrondrial Membrane Cytochrome b_5 Using Direct and Mediated Electrochemistry: Comparison of Results

Purpose

There is considerable interest in elucidating the factors that affect the redox potential of cytochromes, as well as other electron transfer proteins (e.g., blue copper and iron-sulfur proteins). In this Capsule, two methods used to measure the redox potential of outer mitochrondrial membrane (OM) cytochrome b_5 are discussed, and the results of the two methods are compared.

Reference

Cation-Promoted Cyclic Voltammetry of Recombinant Rat Outer Mitochrondrial Membrane Cytochrome b_5 at a Gold Electrode Modified with b-Mercaptopropionic Acid, M. Rivera, M.A. Wells. and F.A. Walker, Biochemistry 33 (1994) 2161-2170.

Method

Although cyclic voltammetry is widely used to study transition metal coordination, early attempts to measure the redox potential of metalloproteins were not successful. This lack of success was attributed to a number of factors, including adsorption and denaturation of the proteins at the electrode surface, and inaccessibility of the coordinated metal ion (which can be buried in the interior of the protein) (1, 2). Indirect electron transfer between the electrode surface and proteins in solution can be achieved using *mediators* to transport electrons. However, the current response to an applied cyclic potential for systems undergoing mediated electron transfer is influenced by a number of factors (e.g., the rate of the second order reaction between the mediator and the protein) in addition to the redox potential of the protein, so the protein redox potential cannot be easily extracted from this current response. An alternative method for measuring redox potentials for mediated systems is to hold the system at a constant potential, and to allow the system to reach equilibrium. The equilibrium behavior is determined by the Nernst equation:

$$E = E^{0'} + \left(\frac{RT}{nF}\right)\ln\frac{C_O}{C_R}$$

which relates the applied potential (E), the formal redox potential ($E^{o'}$) and the concentrations of the oxidized and reduced protein (C_O and C_R, respectively),which can be measured spectroscopically using a spectroelectrochemical cell. This is a spectroscopic cell that contains an Optically Transparent Thin Layer (working) Electrode (OTTLE) that can be either a fine metallic mesh, a thin metallic layer, or an optically transparent material (3). A series of potentials is applied to the cell, ranging from values at which only one oxidation state is present to values at which all the protein is in a different oxidation state, and the applied potential is plotted as a function of the log of the ratio of the equilibrium concentrations. This plot has a slope of 59/n mV (n = number of electrons transferred), and an intercept of $E^{o'}$.

More recently, non-mediated (direct) electron transfer between a protein and an electrode surface has been achieved using modified electrode surfaces. For example, it was shown that a quasi-reversible cyclic voltammogram could be obtained for cytochrome *c* at a gold electrode covered with a monolayer of adsorbed pyridine (4). It was proposed that the hydrogen bonding between bipyridine molecules adsorbed to the electrode surface and lysine ($-NH_3^+$) groups on the electrode surface in the area where the active site was exposed hold the protein close to the electrode surface in an orientation that allows rapid electron transfer between the electrode and the active site. This method of using functional groups (*promoters*) on the electrode surface to bind proteins using electrostatic interactions has been extended to other proteins (1, 2), and it has been suggested that these interactions are similar to those that occur in protein-protein complexes involved in physiological electron transfer reactions. It is important to note these promoters are not mediators; that is, they do not act as intermediates for electron transport.

Results

In this study, both of the above methods were used to measure the redox potential of outer mitochondrial

membrane (OM) cytochrome b_5. The equilibrium spectroelectrochemical measurements were performed using $[Ru(NH_3)_6]^{3+}$] (0.4 mM) and methyl viologen (1 mM) as mediators. A redox potential of 102 mV (vs. NHE) was obtained by this method.

Cyclic voltammograms of cytochrome b_5 were obtained using a 1 mM gold electrode modified with β-mercaptopropionate in the presence of multivalent cations (**F1**). The β-mercaptopropionate has a sulfur

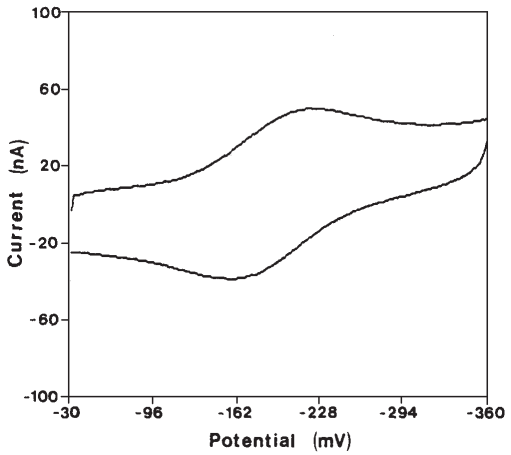

Figure 1. *Cyclic voltammogram of rat outer mitochrondrial cytochrome b_5 (0.1 mM) in 100 mM K-HEPES, pH 7.0, and 0.2 mM poly-L-lysine. Working electrode gold electrode modified with β-mercaptopropionic acid, scan rate = 30 mV s^{-1}. All potentials measured with respect to Ag/AgCl. Reprinted with permission from primary Reference. Copyright 1994 American Chemical Society.*

group at one end which adsorbs to the electrode surface, and a negatively charged carboxylate group at the other end, which is involved in electrostatic interactions with the cytochrome b_5. However, cyclic voltammograms run using the β-mercaptopropionate-modified gold electrode showed no faradaic response, since there are repulsive interactions between the carboxylate groups and the negatively charge protein. Multivalent cations (e.g., poly-L-lysine, Ca^{2+}, Mg^{2+}, and $[Cr(NH_3)_6]^{3+}$] had to be added to the solution in order to obtain a faradaic current (no faradaic current was obtained in the presence of monovalent cations).

It was proposed that a ternary complex involving the cations, the carboxylate groups, and the protein was required in order to obtain electron transfer (**F2**). A similar model had been previously proposed for the interaction between other negatively charge electron transfer proteins (5). However, in contrast to this previous report, it was shown that the redox potential of cytrochrome b_5 varied with the nature and the concentration of the multivalent cation. For example, if the ratio of poly-L-lysine to cytochrome b_5 was increased, the redox potential shifted to more positive values until it leveled off at +8 mV (vs. NHE) when the ratio was 2:1. The subsequent addition of Ca^{2+} or Mg^{2+} resulted in a negative shift of the redox potential until a limiting value of -40 mV was obtained when the concentration of Ca^{2+} or Mg^{2+} was about 85 mM. In the absence of poly-L-lysine, a redox potential of -46 mV was obtained in the presence of Ca^{2+} or Mg^{2+} at concentrations of 40 mM or higher. In contrast, a concentration of only 0.2 mM $[Cr(NH_3)_6]^{3+}$] was required to obtain a faradaic current, and a redox potential of -78 mV was recorded.

The variation in the redox potentials obtained in the cyclic voltammetry experiments suggests that the redox potential is sensitive to the precise nature of the ternary complex required to obtain electron transfer. Since different interactions are involved between the protein and the mediator in the spectroelectrochemical experiment, it is not surprising that different results are obtained from the two methods.

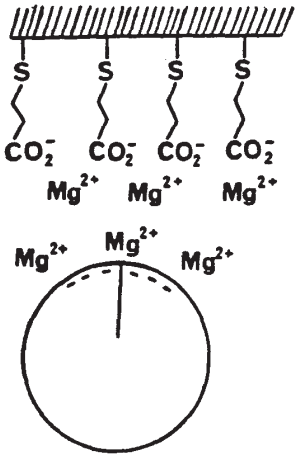

Figure 2. *Model of ternary complex between β-mercaptopropionate adsorbed to the surface of a gold electrode, multivalent cations (e.g., polylysine), and negatively charge cyctochrome b_5. Reprinted with permission from primary Reference. Copyright 1994 American Chemical Society.*

References

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