THE OXIDATION-REDUCTION POTENTIAL OF THE COPPER SIGNAL IN PIGEON HEART MITOCHONDRIA

M. ERECIŃSKA, B. CHANCE and D.F. WILSON
Department of Biophysics and Physical Biochemistry, Johnson Research Foundation, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA

Received 3 June 1971

1. Introduction

The thermodynamic properties of the mitochondrial respiratory chain are of the utmost importance for an ultimate understanding of the mechanism of electron transfer and oxidative phosphorylation [1, 2]. A systematic study has been undertaken in this laboratory to define the equilibrium oxidation–reduction properties of the components of the respiratory chain [3–7]. The present paper is a continuation of these efforts and it reports the oxidation–reduction midpoint potential value of the component responsible for the 830 nm absorption band in pigeon heart mitochondria; this band has been previously identified as due to a copper of cytochrome oxidase [8–11]. The data presented are relevant to the results of Tsudzuki and Wilson [12] on isolated cytochrome oxidase as well as to those of Wilson et al. [13] on the EPR determined midpoint potential of copper signal in intact pigeon heart mitochondria.

2. Materials and methods

Pigeon heart mitochondria were isolated according to the method of Chance and Hagihara [14]. Oxidation–reduction titrations of the mitochondrial copper were performed in the equipment designed by Dutton [15] which permits simultaneous potential (calomel and platinum electrodes) and absorbance measurements under strictly anaerobic conditions. The anaerobic cuvette is continuously flushed with helium. Any oxygen present in the aqueous phase is removed by respiration via endogenous substrate or by addition of small aliquots of NADH. Removal of oxygen resulted in anaerobiosis evidenced by a drop in oxidation–reduction potential to less than 250 mV.

The redox mediators used in the present study were 40 μM phenazinemethosulphate (E'_0 + 80 mV) and 40 μM diaminodurene (E'_0 = + 240 mV); both are two electron acceptors (n value) [16]. Oxidation–reduction potentials were made more positive by addition of potassium ferricyanide and more negative by the addition of NADH. In some experiments (at higher pH values) the presence of endogenous substrate caused a slow negative drift in the oxidation–reduction potential rendering external addition of reductant unnecessary. The oxidation–reduction state of copper was measured in a special dual wavelength spectrophotometer by the absorbancy changes at 840–1000 nm. At these wavelengths interference filters are more efficient than monochromators since the absorption bands of the copper components of the mitochondria are relatively broad [17]. The photodetector was an RCA electron photomultiplier (7102).

Protein was measured by the biuret method [18].

3. Results

The results are plotted as the logarithm of the ratio of the oxidized and reduced form (abscissa) against the observed oxidation–reduction potential (ordinate) (E_h) on the hydrogen scale. In this way, according to the Nernst equation we could expect for a single electron transferring component (n=1) a linear curve with a slope of 59.3 mV per log decade. As shown in

North-Holland Publishing Company – Amsterdam

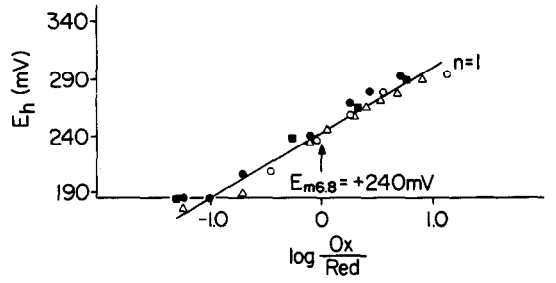

Fig. 1. The oxidation–reduction potential of the 840–1000 nm signal of intact pigeon heart mitochondria. The mitochondria (12 mg prot./ml) were suspended in 0.225 M mannitol–0.075 M sucrose–0.050 M morpholinopropane sulphonate (MOPS) buffer, pH 6.8. The redox mediators used are described under methods. (•) reductive titration, (▲) oxidative titration, (■) reductive titration in the presence of ATP, (○) reductive titration in the presence of 15 μM pentachlorophenol. The line is for theoretical n=1.

fig. 1, the titration points follow a theoretical line with an n value of 1 which has a midpoint potential of +240 mV at pH 6.8.

Oxidation–reduction titration performed on tightly coupled mitochondria was repeated in the presence of 1 mM ATP and the curve obtained (solid squares) was found indistinguishable from that in the absence

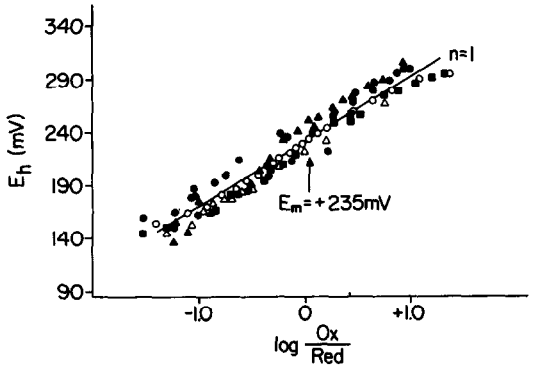

Fig. 2. pH dependence of the 840–1000 nm signal of pigeon heart mitochondria. The mitochondria (6 mg prot/ml) were suspended in 0.225 M mannitol–0.075 M–sucrose, 0.05 M MOPS buffer (pH 6.5, 7.0 and 7.5) or 0.05 M tris (pH 8.0 and 8.5). The redox mediators used are described under methods. (•) pH 6.5, (▲) pH 7.0, (■) pH 7.5, (○) pH 8.0 and (△) pH 8.5. The same symbols are used for oxidative and reductive titrations.

of ATP (solid circles and open triangles) or in the presence of 15 μM pentachlorophenol (open circles). Since pH dependence of the midpoint potential of an oxidation–reduction component can provide evidence for or against a direct coupling of an acid or base group to the oxidation–reduction process the titration was performed at five pH values in the range 6.5 to 8.5 (fig. 2). The midpoint potential of the 830 nm band is pH independent in the pH range 6.5–8.5 and the mean midpoint potential value is +235 mV (±10 mV), identical to that of fig. 1.

4. Discussion

It has been known since the early studies of Griffiths and Wharton [8–9] and those of Beinert [10, 11] that the 830 nm absorption band as well as the EPR copper signal have been associated with one of the two copper atoms of the cytochrome oxidase. These findings were further confirmed by the data of Tzagoloff and Mac Lennan [19] in which the oxidation reduction potential of the 830 nm band of the isolated oxidase was determined from the equilibrium values of the relative states of reduction of the α band of cytochrome *c*. The absorbing component had the midpoint potential of +284 mV and an n value of 1. Tsudzuki and Wilson [12], using the potentiometric technique of Dutton [15], titrated the 830 nm band of the isolated beef heart oxidase and found it to have an n value near to 1 and a midpoint potential of +225 mV; oxidation–reduction titration of the characteristic EPR signal gave a midpoint potential value of +225 mV. In addition the EPR signal of the copper in pigeon heart mitochondria was found by Wilson et al. [13] to have an n value of 1 and a midpoint potential of +250 mV.

The present communication affords a direct comparison between the 830 nm titrated copper signal and the data obtained using EPR technique. The midpoint potential values obtained by both methods are nearly identical: +240 mV by absorbance measurement and +250 mV by EPR technique. Furthermore, it was found that similarly to cytochrome *a*, *c* and c_1, but in contrast to cytochromes a_3 and b_T, midpoint potential value of copper appears to be unaffected by ATP.

The pH independence of the midpoint potential of copper moiety provides evidence against a coupling of an acid or base group to the measured oxidation–reduction process [16].

References

[1] D.F. Wilson, P.L. Dutton and B. Chance, Energy Transduction in Respiration and Photosynthesis (Adriatica Editrice, Bari, 1971).

[2] B. Chance, D.F. Wilson, P.L. Dutton and M. Erecińska, Proc. Natl. Acad. Sci. U.S. 67 (1970) 560.

[3] D.F. Wilson and P.L. Dutton, Arch. Biochem. Biophys. 136 (1970) 583.

[4] D.F. Wilson and P.L. Dutton, Biochem. Biophys. Res. Commun. 39 (1970) 59.

[5] P.L. Dutton, D.F. Wilson and C.P. Lee, Biochemistry 9 (1970) 5077.

[6] M. Erecińska, D.F. Wilson, Y. Mukai and B. Chance, Biochem. Biophys. Res. Commun. 41 (1970) 386.

[7] D.F. Wilson, M. Erecińska, P.L. Dutton and T. Tsudzuki, Biochem. Biophys. Res. Commun. 41 (1970) 1273.

[8] D.E. Griffiths and D.C. Wharton, J. Biol. Chem. 236 (1961) 1850.

[9] D.E. Griffiths and D.C. Wharton, J. Biol. Chem. 236 (1961) 1857.

[10] R.H. Sands and H. Beinert, Biochem. Biophys. Res. Commun. 1 (1959) 175.

[11] H. Beinert and G. Palmer, J. Biol. Chem. 239 (1964) 1221.

[12] T. Tsudzuki and D.F. Wilson, Arch. Biochem. Biophys. (1971) in press.

[13] D.F. Wilson, J.S. Leigh Jr., G. Lindsay and P.L. Dutton, in: II Symp. on Oxidases and Related Redox Systems, eds. T.E. King, H.S. Mason and M. Morrison (Wiley, New York, 1971).

[14] B. Chance and B. Hagihara, in: Proc. Vth Intl. Congress Biochem., eds. A.N.M. Sissakian, Vol. 5 (Pergamon Press, New York, 1963) p. 3.

[15] P.L. Dutton, Biochim. Biophys. Acta 226 (1971) 63.

[16] M.W. Clark, Oxidation–Reduction Potentials of Organic Systems (Waverly Press, Baltimore, 1960).

[17] B. Chance, in: Biochemistry of Copper, eds. J. Peisach, P. Aisen and W.E. Blumberg (Academic Press, New York, 1966) p. 293.

[18] A.G. Gornall, C.S. Bardawill and M.N. David, J. Biol. Chem. 177 (1949) 751.

[19] A. Tzagoloff and D.H. MacLennan, in: Biochemistry of Copper, eds. J. Peisach, P. Aisen and W.E. Blumberg (Academic Press, New York, 1966) p. 253.