REDOX BOHR-EFFECTS IN THE CYTOCHROME SYSTEM OF MITOCHONDRIA

S. PAPA, F. GUERRIERI and G. IZZO
Institute of Biological Chemistry, Faculty of Medicine, University of Bari, Bari, Italy

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1. Introduction

Redox titrations show that the midpoint potential (E_m) of various electron carriers in coupling membranes decreases as the pH of the reaction medium is raised within certain ranges [1]. As far as the cytochrome system of mitochondria is concerned the *b* cytochromes [2–4], the heme groups of cytochrome *c* oxidase [5–7] and the Rieske Fe–sulfur center, *g* 1.90 [8] have been shown to exhibit such a property.

This feature indicates the occurrence in electron carriers of cooperative linkage between electron transfer by the metal and proton transfer by ionizable groups, possibly in the apoprotein, owing to increase of their pK upon reduction ([1,3,9] cf. [10]). Thus at pH values in the order of the pK_a values oxidation of the electron carrier should result in the release of protons and its reduction in the uptake of protons [9,11].

These considerations, as well as the inherent ambiguities of redox titrations of membrane bound systems [10], has led us to investigate these linkage phenomena – denominated, by analogy to those described for hemoglobin [12,13], redox Bohr effects or membrane Bohr effects [14] – by an independent approach based on direct measurements of scalar proton release and uptake associated to redox transitions of electron carriers in the respiratory chain of mitochondria.

The results presented here show that this approach provides direct demonstration for the occurrence of redox Bohr effects in the cytochrome system and is particularly suitable for their characterization.

2. Methods

Heavy beef-heart mitochondria were prepared as in [15]. Oxido-reduction of cytochromes and pH changes were monitored simultaneously on the same sample of mitochondrial suspension in a thermostated (±0.1°C) spectrophotometric cuvette under continuous stirring and a stream of nitrogen.

Oxido-reductions were monitored with a Johnson Foundation dual wavelength spectrophotometer. The pH of the suspension was monitored with a combination electrode connected to a differential electrometer amplifier. The potentiometric deflections were calibrated by double titration with standard HCl and KOH. The circuit used allowed the pH to be measured with a precision of 0.001 pH unit [16].

3. Results and discussion

Figure 1 illustrates the characteristics of proton transfer associated to redox transitions of terminal electron carriers caused by repetitive oxygen pulses of anaerobic beef-heart mitochondria supplemented with antimycin and FCCP. In the presence of this proton-conducting uncoupler vectorial proton translocation is abolished and net pH changes resulting from scalar proton transfer reactions can be measured.

Aerobic oxidation of cytochrome a,a_3 and *c* cytochromes was accompanied by reduction of *b* cytochromes (cf. [17,18]). Enough oxygen was administered so to ensure complete oxidation of cytochromes *c* and cytochrome oxidase and full pH equilibration by FCCP between the mitochondrial matrix and the outer phase.

Aerobic oxidation of electron carriers should result in the consumption of a stoicheiometric amount of protons for reduction of O_2 to H_2O and their re-

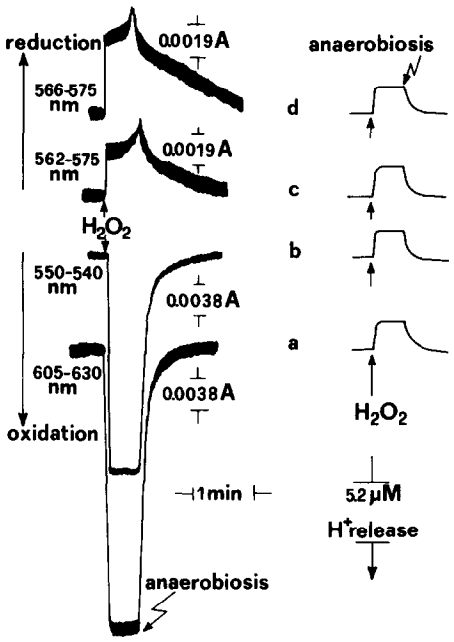

Fig.1. Redox transitions of terminal electron carriers and associated scalar proton transfer reactions induced by oxygen pulses of anaerobic beef-heart mitochondria. Mitochondria 2 mg protein/ml were incubated in: 200 mM sucrose, 30 mM KCl, 5 mM K-succinate, 2 μg/mg protein oligomycin, 0.5 μg/mg protein valinomycin, 0.5 μg/mg protein rotenone, 1 μg/mg protein antimycin, 3 μM FCCP and 0.01 mg/ml catalase. Final pH 7.4. Temperature 25°C. Oxygenation was brought about by repetitive additions of 5 μl 0.1% H_2O_2 to anaerobic mitochondria. The mitochondrial suspension was made anaerobic by succinate oxidation under a stream of nitrogen. Antimycin was then added and the suspension repetitively pulsed with H_2O_2. The oxygen made available was exhausted in about 30 s by the slow antimycin resistant respiration.

reduction by hydrogenated endogenous reductants in the production of the same amount of protons. The data obtained from the measurements illustrated in fig.1 show that the net alkalinization produced by oxidation of electron carriers was, in fact, equivalent to the acidification ensuing upon their anaerobic re-reduction (see also table 1). Proton uptake and release were, however, considerably lower than the sum of the electron carriers undergoing a cycle of oxidation and reduction (table 1). This difference, which is practically due to proton release from terminal electron carriers associated to their aerobic oxidation and proton uptake upon their re-reduction, provides an overall estimate of net Bohr effects in this segment of the respiratory chain.

The overall number of Bohr protons per electron transferred to oxygen which could be directly calculated in this way amounted at pH 7.4 to 0.63 (table 1). It is, however, possible to calculate more closely the contribution to Bohr protons of the various redox transitions elicited by oxygenation.

Since the reducing equivalents for the small reduction of cytochromes *b* are provided by ubiquinol [11,17,18,24] this process should contribute stoicheiometric proton release. This acidification is, however, in part neutralized (by 73% at pH 7.4) by reductive protonation of cytochromes *b* [9,11].

Also cytochrome *c* exhibits linkage between electron and proton binding; however this phenomenon is significant only at rather alkaline pH values [25]. At pH 7.4 $<$ 1% of oxido-reduction of cytochrome *c* is proton coupled.

Correction of the overall net estimate of Bohr protons for proton release associated to reduction of cytochromes *b* by ubiquinol and oxidation of cytochrome *c* provides the estimate for proton transfer due to redox Bohr effects in cytochrome oxidase and the redox centers of the $b.c_1$ complex which are oxidized by oxygen in the presence of antimycin. The known redox components which behave in this way are cytochromes c_1 and the Rieske Fe–sulfur center. The overall proton electron stoicheiometry number (s/n) calculated for these redox Bohr effects amounted at pH 7.4 to 0.8 and varied from 0.7–0.8 at the pH 6.2–9.1 (fig.2).

This means that at physiological pH values 70% and more of the oxidations of the redox centers of cytochrome oxidase and the $b.c_1$ complex is associated to proton release from their ionizable groups.

The redox titration data available for cytochrome oxidase [5–7] indicate positive cooperativity between electron and proton transfer for the heme groups in the high potential state. The copper, on the other hand, appears silent in this respect [1,6]. The situation for the low potential heme is not clear [1,5–7]. Furthermore these redox titrations do not provide consistent data as far as the pH range and the slope for the E_m/pH dependence curve is concerned and consequently do not allow us to obtain conclusive information on the actual pK_a values

Table 1
Analytical evaluation of proton transfer reactions associated to redox transitions of terminal electron carriers caused by oxygenation of anaerobic beef-heart mitochondria in the presence of antimycin and FCCP

Proton transfer reactions (ng ions H^+ .mg protein^{-1})		Respiratory carrier redox transitions (nmol .mg protein^{-1})	
		Oxidation–reduction	
H^+ uptake	1.31 ± 0.050	Hemes a,a_3	1.26 ± 0.02
H^+ release	1.31 ± 0.050	Cu	1.26 ± 0.02
		Cytochromes c,c_1	0.69 ± 0.02
		Fe–S protein (g 1.90)	0.34 ± 0.01
Bohr H^+ (Σe^- flow – H^+ uptake)	2.24 ± 0.123	Σe^- flow	3.55 ± 0.07
Bohr $H^+/\Sigma e^-$	0.63 ± 0.02		
		Reduction–oxidation	
H^+ release for cytochromes b reduction by QH_2	0.045 ± 0.001	Cytochrome b	0.165 ± 0.004
H^+ release for cytochrome c oxidation	0.002 ± 0.00006		
Bohr $H^+/\Sigma e^-$ (corrected)	0.68 ± 0.02		

For experimental conditions and procedure see legend to fig.1. The nmoles of hemes a and a_3 were calculated by using a $\Delta\epsilon$ mM at 605–630 nm of 14 [19], cytochromes c with a $\Delta\epsilon$ mM at 550–540 nm of 19.1 [20]. The atoms of copper oxidized were taken as equivalent to the moles of $a + a_3$ oxidized [21]; the Fe–sulfur center, g = 1.90 as equivalent to half the amount of cytochromes c oxidized [22]. The nmoles of cytochrome b reduced were calculated by assuming a contribution of b_{566} to $\Delta A_{566-575}$ of 60% and that of b_{562} at 562–575 nm of 75% [18,22,23]. The $\Delta\epsilon$ mM of b_{566} at 566–575 nm was taken as 16 and that of b_{562} at 562–575 nm as 14.6 [23]. The corrected Bohr $H^+/\Sigma e^-$ was obtained by subtracting from the estimate of Bohr H^+ given at line 3, H^+ release associated to reduction of cytochromes b by QH_2 [11] and oxidation of cytochrome c [25]; the nmoles of cytochrome c oxidized (0.34 nmol .mg protein^{-1}) were subtracted from the Σe^- flow. The data reported are the means of 3 expt. ± SEM

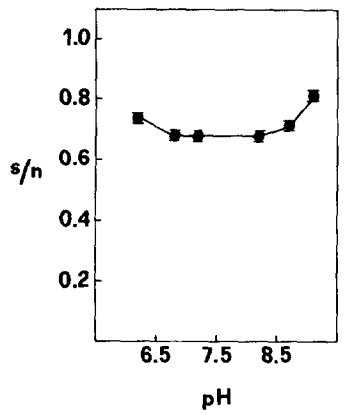

Fig.2. pH dependence of the H^+/e^- stoicheiometry number (s/n) for redox Bohr effects in the cytochrome system of beef-heart mitochondria. For experimental details see legends to fig.1 and table 1. The points represent the mean of 3 experiments ± SEM.

of the oxidized and reduced redox carriers and the proton/electron stoicheiometry number (s/n). It is conceivable that further application of the approach presented here to redox carriers in the mitochondrial membrane, as well as to isolated cytochrome *c* oxidase and the $b.c_1$ complex, can produce this and other information, needed to define the characteristics of linkage between electron and proton transfer in electron carriers and to shed light on its role in electron transport and energy transduction (see [9,14,26]).

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