ON THE PROTON TRANSLOCATION SYSTEM OF THE INNER MITOCHONDRIAL MEMBRANE

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1. Introduction

Oxygen or ATP pulses cause a reversible pH decrease in mitochondrial suspension [1, 2]. In submitochondrial particles, obtained by sonication and consisting of vescicles of the inner mitochondrial membrane turned "inside out" [3], respiration or ATP hydrolysis causes proton movements in the *reverse* direction [4–6]. This inversion of polarity, and observations on intramitochondrial pH changes [7–9] indicate that the energy-linked pH changes represent, at least in part, *effective* translocation of protons across the inner mitochondrial membrane.

Potassium salts stimulate the respiration-driven proton uptake in sonic particles [6]. This effect is potentiated by valinomycin, indicating that, at least in the presence of this antibiotic, the stimulation of proton uptake is due to K^+ translocation (cf. [10]). Since, however, the activity of K^+-salts varies with the anions used, these must also be involved. In this paper a study of the effect of a series of salts on proton translocation in submitochondrial particles, is presented.

2. Experimental

Mg-ATP sonic particles from beef heart mitochondria were prepared according to Löw and Vallin [11]. The pH of the suspension was measured with a highly sensitive pH meter with a low-resistance "Ingold" glass electrode (response time about 50 msec). The pH changes were quantitated in terms of proton equivalents by double titration with standard solutions of KOH and HCl both in the anaerobic and the aerobic state. Respiration was measured with a Clark electrode or a vibrating plantinum electrode.

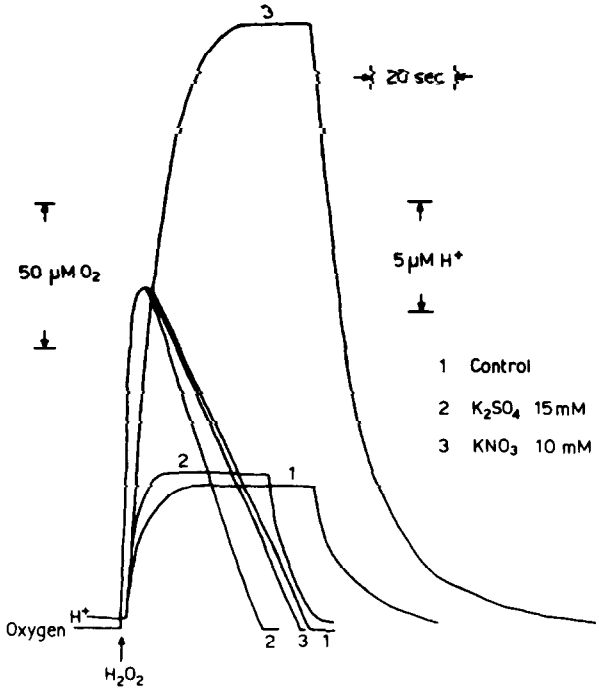

Fig. 1. Effect of KNO_3 and K_2SO_4 on proton translocation and respiration during oxygen pulses in submitochondrial particles. The reaction mixture (final vol. 2.1; final pH 7.5) contained 250 mM sucrose 15 mM K-succinate, 0.4 mg purified catalase and 3.3 mg particle protein. Respiration was started by adding 10 μl of 0.2% H_2O_2 to anaerobic particles. Temperature 25°. Respiration was recorded, simultaneously with pH, by a Clark electrode.

Table 1
Effect of salts on proton and electron flow in sonic submitochondrial particles

Salts	Concn. (mM)	Stimulation initial rate H^+ uptake (%)	Extent H^+ uptake (ngion/mg prot)	Velocity constant H^+ efflux (sec^{-1})	Stimulation initial rate H^+ efflux (%)	Stimulation or inhibition steady-state respiration (%)	Steady-state H^+/O
Control			5.00	0.590			0.50
KCl	15	37	6.69	0.252	15	+4	0.55
KNO_3	10	70	18.40	0.154	58	+3	0.77
KI	10	126	28.82	0.134	70	−22	1.09
KSCN	1	210	23.75	0.283	204	−3	1.55
Na-TPB	0.05	155	15.90	0.154	115	+7	1.00
K_2SO_4	15	66	5.25	0.866	50	+27	0.59
K-Acetate	5	93	4.50	0.845	30	+30	0.50

Temp., 25°; pH, 7. For other experimental details see Experimental Section and legend to fig. 1. Na-TPB: Na-tetraphenylborate. The salts were added at concentrations giving about half-maximal stimulation of the initial rate of proton uptake.

3. Results

Oxygen pulses in succinate-supplemented particles induced abrupt uptake of protons by the particles, followed by a stationary phase (fig. 1). With anaerobiosis, an exponential release of protons occurred which proceeded according to a first order equation (see fig. 2). It is assumed [12, 13] that, in the stationary state, the rate of proton influx is equal to that of proton efflux. The latter is given by the initial rate of proton efflux in anaerobiosis [13].

Table 1 summarizes the effects of salts on: the initial rate and the extent of proton uptake; the velocity constant of proton efflux in anaerobiosis; the rate of electron and proton flow in the steady-state and the H^+/O ratio. Chloride, nitrate, iodide, thiocyanate and tetraphenylborate caused to different extents, stimulation of the initial rate and the extent of respiration-driven proton uptake. This was accompanied by stimulation of the initial rate of the anaerobic efflux of protons but by a decrease of the velocity constant of this process (see fig. 2). At the concentrations used these salts, except KI which caused inhibition, had practically no effect on respiration. This resulted in an increase of the steady-state H^+/O ratio.

Sulfate and acetate increased the initial rate of proton uptake, the initial rate and the velocity constant of proton efflux (see fig. 2). The extent of proton uptake was not significantly affected by these salts and respiration was stimulated.

Table 2
Effect of KNO_3 and $NaNO_3$ on proton translocation in sonic submitochondrial particles

Salts (20mM)	Stimulation initial rate H^+ uptake (%)	Extent H^+ uptake (ngion/mg prot)	Stimulation initial rate H^+ efflux (%)	Velocity constant H^+ efflux (sec^{-1})
KNO_3	50	41.32	63	0.087
$NaNO_3$	47	20.04	113	0.154

For details see Experimental and fig. 1. pH: 7.

Table 2 shows that $NaNO_3$ gave a higher velocity constant of proton efflux (see fig. 2) but a smaller extent of proton uptake than KNO_3.

4. Discussion

The stimulation of the initial rate of proton uptake

by the salts appears to be related to the permeability of artificial phospholipid membranes [14–16] and mitochondrial membrane [17] to the anionic species. Nitrate, iodide, thiocyanate and tetraphenylborate appeared to increase the steady-state turnover of protons, without stimulating respiration. This observation can be explained in terms of a proton pump [2, 9] of electrogenic nature [9]; see however [7, 18]. In this case the thermodynamic potential difference of protons across the membrane consists of a chemical (Δ pH) and an electrical ($\Delta\Psi$) component. Both exert a back-pressure on proton uptake and drive proton efflux. A salt, which dissipates the $\Delta\Psi$ through distribution of the anion in the electric field, stimulates proton uptake and converts the proton efflux driven by the electric field (probably not seen by the electrode, due to the short time life of the electric field [13]) into a pure diffusion. Thus the increase of the proton turnover would be, for a large part, only apparent. Consistent with this explanation is also the fact that these salts caused a decrease of the velocity constant of proton efflux. When the $\Delta\Psi$ is dissipated the diffusion of protons, in anaerobiosis, becomes electrogenic and depends upon the compensatory movement of other ions. $NaNO_3$ gave a higher velocity constant of proton efflux than KNO_3. This would suggest that the Δ pH-driven proton efflux is, at least in part, coupled to a counterflux of cations (see [9]). In favour of an electrogenic proton pump is also the fact that respiration causes accumulation of the anions by the particles [16]. We have found [19] that respiration caused accumulation of ^{14}C-thiocyanate by the particles which was partly depressed by valinomycin and completely suppressed by uncouplers or lytic detergents.

The increase of the velocity constant of proton efflux given by sulfate and acetate is possibly due to back-diffusion of the acid. Thus these salts give a net increase of energy-expending proton turnover across the membrane.

An electrogenic proton pump could be either directly coupled to electron flow [9] or driven by high-energy intermediates. This problem as well as the possibility of additional interaction of the salts with the pump are beyond the scope of the present paper.

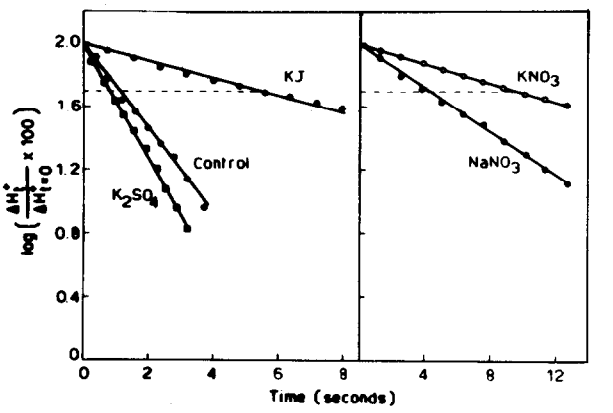

Fig. 2. Effect of salts on velocity constant of anaerobic efflux of protons. For experimental conditions see fig. 1 and Experimental section. pH: 7.

Acknowledgements

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