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Respiration driven Cl^- uptake by submitochondrial particles

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Both Mg^{2+} and oligomycin are required for the establishment of a membrane potential and the uptake of Cl^- in submitochondrial particles prepared from rat liver. The effect of oligomycin is considered to be due to blocking of H^+ conduction through exposed F_0 channels of the ATPase complex whereas Mg^{2+} may more directly affect the anion-conducting channel.

Anion channel; Submitochondrial particle; Membrane potential; Mg^{2+}; Oligomycin

1. INTRODUCTION

Previously we have reported the existence of an electrogenic pH-dependent anion uniport in rat liver mitochondria [1], the properties and possible functions of which have been reviewed by Garlid and Beavis [2]. We have also reported a direct method for investigating the anion uniport by measuring $^{36}Cl^-$ accumulation by submitochondrial particles [3].

Sorgato et al. [4] have shown that it is possible to determine the membrane potential in submitochondrial particles by examination of the distribution of the freely permeant anion CNS^- across the submitochondrial particle membranes. Using this procedure we have investigated the effects of Mg^{2+} concentration, oligomycin concentration and addition of the protonophore FCCP on the respiration-driven membrane potential and $^{36}Cl^-$ accumulation in submitochondrial particles.

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Abbreviations: FCCP, carbonyl cyanide 4-(trifluoromethoxy)-phenylhydrazone; Hepps, *N*-2-hydroxyethylpiperazine-*N'*-3-propanesulphonic acid

2. EXPERIMENTAL

2.1. *Materials*

$Na^{36}Cl$, $K^{14}CNS$, $[^3H]H_2O$ and [U-^{14}C]sucrose were obtained from Amersham (Aylesbury, England). All other chemicals used were of the purest grade available.

2.2. *Preparation of mitochondria and submitochondrial particles*

Mitochondria were prepared from adult male rats according to Selwyn et al. [1].

Submitochondrial particles were obtained from rat liver mitochondria by modifications of the procedure originally described by Gregg [5]. Controlled sonication of rat liver mitochondria (70–80 mg/ml) was carried out in a hypotonic buffer (50 mM sucrose, 9 mM Hepes-KOH, pH 7.4) for 5 min at nominally 30 W. Buffer temperature was maintained at 4°C using an ice/water cooling bath and further, the sonicator used (Heat Systems ultrasonic processor soni-probe 375) was set to a 50% pulse cycle to facilitate cooling. Protein concentrations were determined by the method of Lowry et al. [6].

2.3. *Determination of the uptake of $^{14}CNS^-$ and $^{36}Cl^-$ by submitochondrial particles*

The method used for the determination of $^{14}CNS^-$ accumulation was as previously described for measurement of $^{36}Cl^-$ accumulation by submitochondrial particles [3]. To monitor $^{14}CNS^-$ uptake by submitochondrial particles $K^{14}CNS$ (0.87 mM, 0.5 μCi/ml final) was used in the incubation in place of $^{36}Cl^-$. $^{36}Cl^-$ concentration was 0.9 mM Cl^- (0.5 μCi/ml final) as in [3].

2.4. *Determination of submitochondrial particle internal volume and membrane potential*

The internal volume of submitochondrial particles was deter-

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mined by a procedure modified from that described by Sorgato et al. [4]. Submitochondrial particles (7.5 mg) were added to buffer (1 ml) containing 250 mM sucrose, 50 mM Hepps-NaOH, pH 8.0, 20 nCi [U-^{14}C]sucrose, 50 nCi [^{3}H]H_2O which was centrifuged (150 000 × g, 60 min). Pellets and supernatants were solubilised in SDS (1% w/v, 0.5 ml). Aliquots of these samples were analysed for [^{3}H]H_2O and [U-^{14}C]sucrose distributions by liquid scintillation counting. Determination of the transmembrane distribution of $^{14}CNS^-$ as described above and knowledge of the internal submitochondrial volume allowed estimation of the membrane potential in submitochondrial particles.

3. RESULTS AND DISCUSSION

Fig.1 shows the accumulation of both $^{36}Cl^-$ and $^{14}CNS^-$ and the effect of the protonophore FCCP on these accumulations. The patterns of uptake of these anions differ in that $^{14}CNS^-$ uptake rapidly reaches a plateau, which corresponds to the membrane potential generated by succinate oxidation-driven H^+ pumping, whereas $^{36}Cl^-$ accumulation is much slower in reaching a steady state. The addition of FCCP has qualitatively identical effects on the accumulation of both anions, causing efflux of

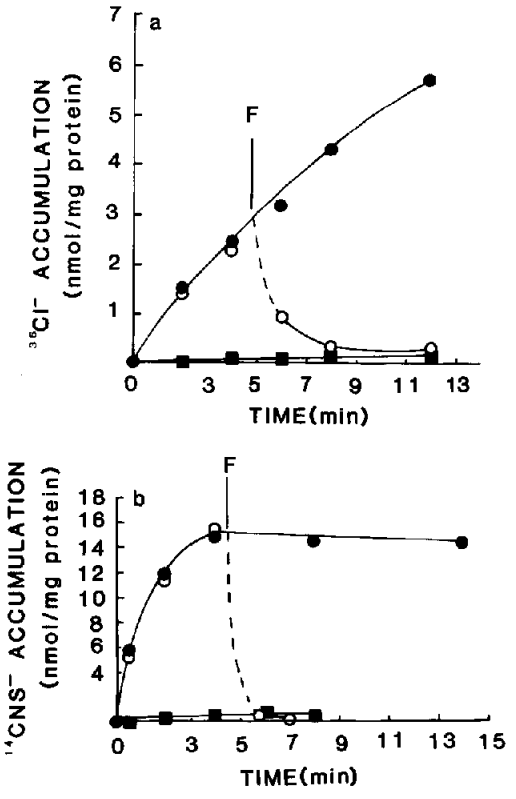

Fig.1. Effect of FCCP on accumulation of $^{36}Cl^-$ and $^{14}CNS^-$ by submitochondrial particles. (a) Effect on $^{36}Cl^-$ accumulation. Incubation conditions as described in section 2. FCCP was added at the time indicated by F. (●) No FCCP added, (○) + FCCP (1 μM final concentration), (■) $^{36}Cl^-$ accumulation in the absence of sodium succinate. (b) Effect on $^{14}CNS^-$ accumulation. Incubation conditions as described in section 2. FCCP was added at the time designated by F. (●) No FCCP added, (○) + FCPP (1 μM final concentration), (■) $^{14}CNS^-$ accumulation in the absence of sodium succinate.

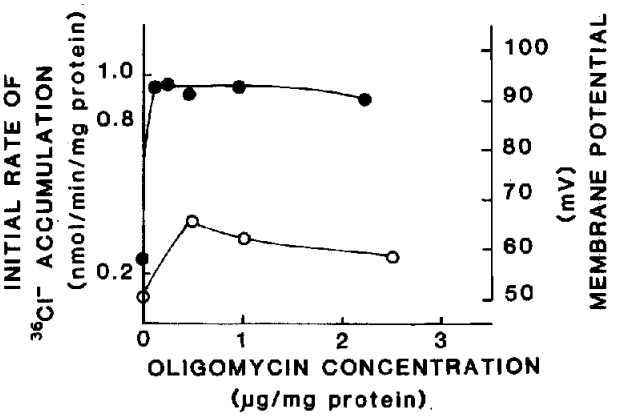

Fig.2. Effect of oligomycin concentration on accumulation of $^{36}Cl^-$ and membrane potential in submitochondrial particles. Incubation conditions were as described in section 2 except that the final Mg^{2+} concentration in these experiments was fixed at 10 mM and added oligomycin concentration was varied. (○) $^{36}Cl^-$ accumulation, (●) membrane potential.

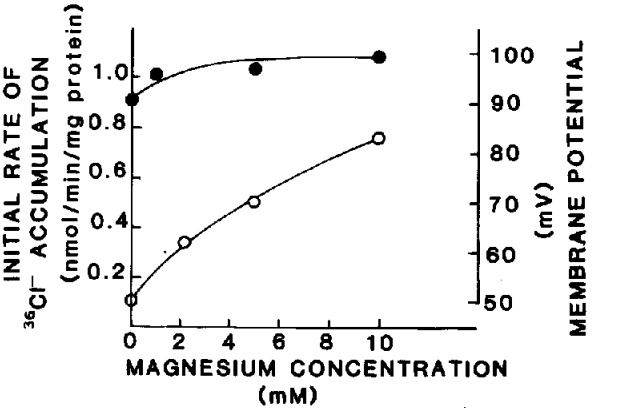

Fig.3. Effect of Mg^{2+} concentration on accumulation of $^{36}Cl^-$ and membrane potential in submitochondrial particles. Incubation conditions were as described in section 2 except that the final oligomycin concentration was fixed at 0.5 μg/mg protein and the added Mg^{2+} concentration was varied. (○) $^{36}Cl^-$ accumulation, (●) membrane potential.

the anions to the levels observed in the absence of succinate. These data therefore indicate that Cl^- uptake is dependent on the proton gradient generated by succinate oxidation.

Fig.2 shows the effect of oligomycin concentration on both the initial rate of $^{36}Cl^-$ accumulation and the membrane potential (estimated from CNS^- distribution and the internal volume of submitochondrial particles). It is observed that oligomycin has different effects on these two parameters. Oligomycin is seen to be required to establish a membrane potential in submitochondrial particles. This is in accord with the observation and suggestion of Mitchell and Moyle [7] that when the F_1 component of the ATPase complex has been detached, during preparation of the submitochondrial particles, the F_0 component acts as a proton-conducting channel but this proton conductance is blocked by oligomycin. At oligomycin concentrations greater than 0.5 μg oligomycin/mg protein the membrane potential is only slightly affected but some inhibition of the initial rate of uptake of $^{36}Cl^-$ is observed. This may be due to a non-specific effect of oligomycin on membrane permeability or may result from effects on counterion movement, leading to a decrease in the internal pH.

Fig.3 shows data for the effect of Mg^{2+} concentration on both the initial rate of $^{36}Cl^-$ accumulation and the membrane potential. The increase in initial rate of $^{36}Cl^-$ uptake produced by Mg^{2+} is much greater than would be predicted from the increase in membrane potential. It is important to note that over this range of Mg^{2+} concentrations the internal volume of submitochondrial particles remains constant (0.513 μl/mg protein in the absence of Mg^{2+} and 0.515 μl/mg protein in the presence of 10 mM Mg^{2+}) and hence increased $^{36}Cl^-$ accumulation is not a result of swelling of the submitochondrial particle.

These are the first reports of Mg^{2+} playing a stimulatory role in relation to anion permeability of the mitochondrial inner membrane and may be contrasted with reports by Garlid [8] and Jung and Brierley [9] who suggest that changes which increase anion permeability do so by decreasing intramitochondrial Mg^{2+}. Also, Sorgato et al. [10], using patch-clamp techniques, have recently demonstrated anion conductance, in mitoplasts from giant mitochondria, in the absence of added external Mg^{2+}. There appear to be two possibilities for the stimulation which we have observed: firstly, that Mg^{2+} stimulates by some indirect action which causes the inside of the submitochondrial particle to become more alkaline thereby increasing the conductance of the pH-dependent anion channel, or that Mg^{2+}, at the matrix face of the mitochondrial inner membrane, has a direct effect on the pH-dependent anion channel.

Thus, we conclude that $^{36}Cl^-$ accumulation by submitochondrial particles can be driven by the membrane potential produced by succinate oxidation. Maximal rates of $^{36}Cl^-$ accumulation require the presence of both oligomycin and Mg^{2+}, the former to make the submitochondrial particles impermeable to protons, the latter possibly having a direct effect on the anion-conducting channel.

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