

# Respiration driven $\text{Cl}^-$ uptake by submitochondrial particles

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Both  $\text{Mg}^{2+}$  and oligomycin are required for the establishment of a membrane potential and the uptake of  $\text{Cl}^-$  in submitochondrial particles prepared from rat liver. The effect of oligomycin is considered to be due to blocking of  $\text{H}^+$  conduction through exposed  $\text{F}_0$  channels of the ATPase complex whereas  $\text{Mg}^{2+}$  may more directly affect the anion-conducting channel.

Anion channel; Submitochondrial particle; Membrane potential;  $\text{Mg}^{2+}$ ; 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  $^{36}\text{Cl}^-$  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  $\text{CNS}^-$  across the submitochondrial particle membranes. Using this procedure we have investigated the effects of  $\text{Mg}^{2+}$  concentration, oligomycin concentration and addition of the protonophore FCCP on the respiration-driven membrane potential and  $^{36}\text{Cl}^-$  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

$\text{Na}^{36}\text{Cl}$ ,  $\text{K}^{14}\text{CNS}$ ,  $[^3\text{H}]\text{H}_2\text{O}$  and  $[\text{U-}^{14}\text{C}]\text{sucrose}$  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 $^{14}\text{CNS}^-$ and $^{36}\text{Cl}^-$ by submitochondrial particles

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

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

The internal volume of submitochondrial particles was deter-

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 Hepes-NaOH, pH 8.0, 20 nCi [ $^{14}\text{C}$ ]sucrose, 50 nCi [ $^3\text{H}$ ]H $_2\text{O}$  which was centrifuged ( $150\,000 \times g$ , 60 min). Pellets and supernatants were solubilised in SDS (1% w/v, 0.5 ml). Aliquots of these samples were analysed for [ $^3\text{H}$ ]H $_2\text{O}$  and [ $^{14}\text{C}$ ]sucrose distributions by liquid scintillation counting. Determination of the transmembrane distribution of  $^{14}\text{CNS}^-$  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  $^{36}\text{Cl}^-$  and

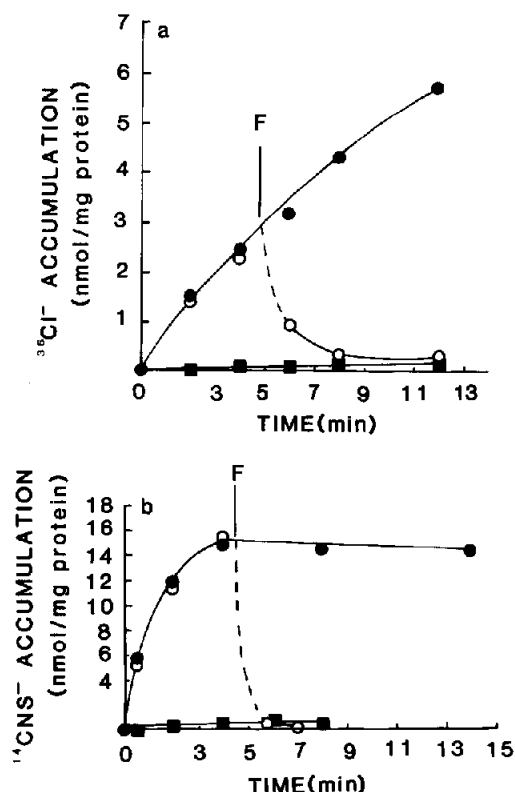


Fig.1. Effect of FCCP on accumulation of  $^{36}\text{Cl}^-$  and  $^{14}\text{CNS}^-$  by submitochondrial particles. (a) Effect on  $^{36}\text{Cl}^-$  accumulation. Incubation conditions as described in section 2. FCCP was added at the time indicated by F. (●) No FCCP added, (○) + FCCP (1  $\mu\text{M}$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

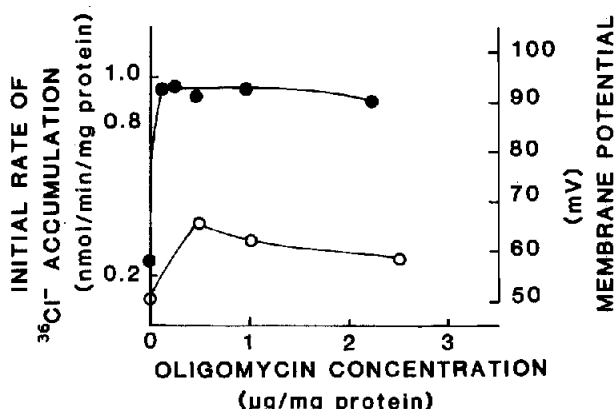


Fig.2. Effect of oligomycin concentration on accumulation of  $^{36}\text{Cl}^-$  and membrane potential in submitochondrial particles. Incubation conditions were as described in section 2 except that the final  $\text{Mg}^{2+}$  concentration in these experiments was fixed at 10 mM and added oligomycin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

$^{14}\text{CNS}^-$  and the effect of the protonophore FCCP on these accumulations. The patterns of uptake of these anions differ in that  $^{14}\text{CNS}^-$  uptake rapidly reaches a plateau, which corresponds to the membrane potential generated by succinate oxidation-driven  $\text{H}^+$  pumping, whereas  $^{36}\text{Cl}^-$  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

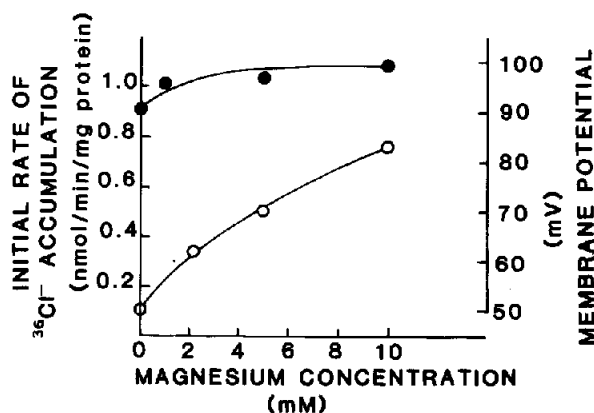


Fig.3. Effect of  $\text{Mg}^{2+}$  concentration on accumulation of  $^{36}\text{Cl}^-$  and membrane potential in submitochondrial particles. Incubation conditions were as described in section 2 except that the final oligomycin concentration was fixed at 0.5  $\mu\text{g}/\text{mg}$  protein and the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

the anions to the levels observed in the absence of succinate. These data therefore indicate that  $\text{Cl}^-$  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  $^{36}\text{Cl}^-$  accumulation and the membrane potential (estimated from  $\text{CNS}^-$  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  $\text{F}_1$  component of the ATPase complex has been detached, during preparation of the submitochondrial particles, the  $\text{F}_0$  component acts as a proton-conducting channel but this proton conductance is blocked by oligomycin. At oligomycin concentrations greater than  $0.5 \mu\text{g}$  oligomycin/mg protein the membrane potential is only slightly affected but some inhibition of the initial rate of uptake of  $^{36}\text{Cl}^-$  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  $\text{Mg}^{2+}$  concentration on both the initial rate of  $^{36}\text{Cl}^-$  accumulation and the membrane potential. The increase in initial rate of  $^{36}\text{Cl}^-$  uptake produced by  $\text{Mg}^{2+}$  is much greater than would be predicted from the increase in membrane potential. It is important to note that over this range of  $\text{Mg}^{2+}$  concentrations the internal volume of submitochondrial particles remains constant ( $0.513 \mu\text{l}/\text{mg}$  protein in the absence of  $\text{Mg}^{2+}$  and  $0.515 \mu\text{l}/\text{mg}$  protein in the presence of  $10 \text{ mM}$   $\text{Mg}^{2+}$ ) and hence increased  $^{36}\text{Cl}^-$  accumulation is not a result of swelling of the submitochondrial particle.

These are the first reports of  $\text{Mg}^{2+}$  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 in-

tramitochondrial  $\text{Mg}^{2+}$ . 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  $\text{Mg}^{2+}$ . There appear to be two possibilities for the stimulation which we have observed: firstly, that  $\text{Mg}^{2+}$  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  $\text{Mg}^{2+}$ , at the matrix face of the mitochondrial inner membrane, has a direct effect on the pH-dependent anion channel.

Thus, we conclude that  $^{36}\text{Cl}^-$  accumulation by submitochondrial particles can be driven by the membrane potential produced by succinate oxidation. Maximal rates of  $^{36}\text{Cl}^-$  accumulation require the presence of both oligomycin and  $\text{Mg}^{2+}$ , 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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