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Effect of oligomygin on proton translocation in submitochondrial particles

In 1965 LEE AND ERNSTER[1] reported that in certain submitochondrial particles critical concentrations of oligomycin restore oxidative phosphorylation, and also cause an inhibition of respiration which is released by uncouplers. The nature of this effect of oligomycin is still not understood (*cf.* refs. 2 and 3). Recently PAPA *et al.*[4,5] found that chloride salts of monovalent cations stimulate respiration and uncouple oxidative phosphorylation in submitochondrial particles (see also ref. 6). Valinomycin potentiates the effect of NH_4Cl and KCl. It has also been observed that valinomycin *plus* nigericin, in the presence of K^+, release the respiratory control induced in submitochondrial particles by oligomycin[7] or dicyclohexylcarbodiimide[3]. Interestingly enough the induction of respiratory control by these substances is accompanied by an energy-linked, nigericin-facilitated, inward translocation of K^+ (refs. 3 and 8). In submitochondrial particles obtained by sonication, respiration (as well as ATP hydrolysis) is accompanied by an inward translocation of protons[9–11]. MITCHELL AND MOYLE[9] have stated that the extent of proton translocation is stimulated (about 25%) by oligomycin. In this paper, it is shown that oligomycin greatly enhances the respiration-linked uptake of protons in submitochondrial particles and depresses the rate of the passive release of protons on the exhaustion of oxygen. The results of this study give further support to the concept that the rate of electron transfer in submitochondrial particles in the absence of phosphate acceptor is controlled by an energy-linked turnover of cations and H^+ across the particle membrane[4,5,12].

Fig. 1A shows the kinetics of proton translocation in EDTA particles[1] during oxygen pulses in the presence of NADH and an NADH regenerating system. The incubation medium contained 30 mM KCl. On initiating respiration by adding H_2O_2 to anaerobic particles in the presence of excess catalase, there was a relatively fast uptake of protons with exponential kinetics which reached a steady state in 20–25 sec. When the particles became anaerobic, the proton gradient decayed exponentially ($t_{\frac{1}{2}} = 2.8$ sec). The addition of 3 μg oligomycin, before the cycle was started again, resulted in inhibition of respiration (from 226 to 169 natoms O_2 per min per mg protein), a substantial increase in the extent of the H^+ uptake, and a decrease in the rate of the passive decay of the H^+ gradient on anaerobiosis ($t_{\frac{1}{2}} = 4.2$ sec). The further addition of valinomycin caused a large increase of the H^+ uptake and some activation of respiration (rate = 196 natoms O_2 per min per mg protein). Finally the addition of nigericin, in the presence of valinomycin, released the inhibition of respiration by oligomycin (rate = 236 natoms O_2 per min per mg protein) and lowered the extent of H^+ uptake below that found in the control.

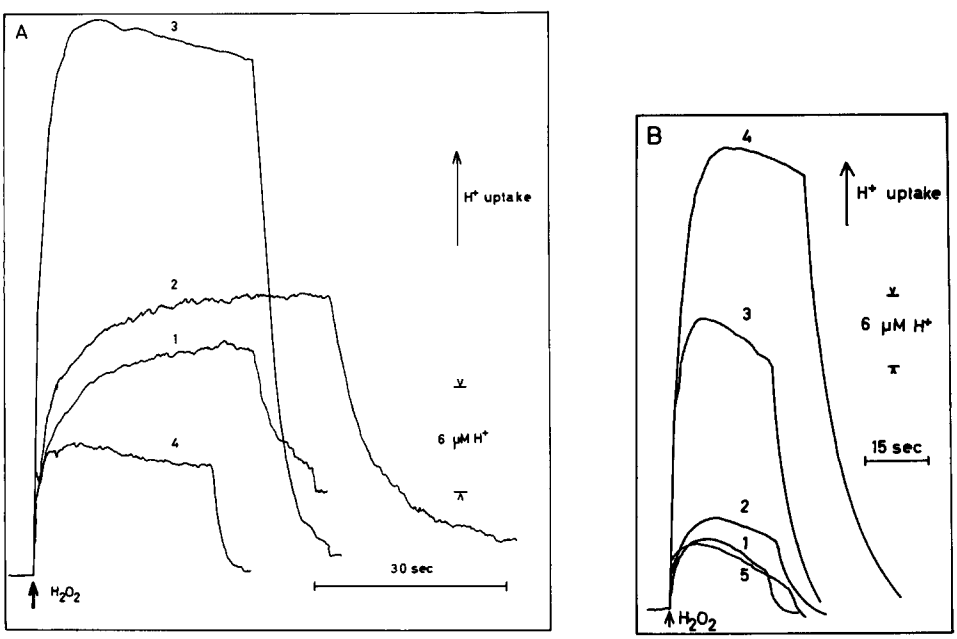

Fig. 1. Effect of oligomycin and valinomycin on proton translocation during oxygen pulses in submitochondrial particles. In the experiment of A the reaction mixture (final vol., 1.5 ml; final pH, 7.5) contained 250 mM sucrose, 1 mM glycylglycine buffer, 30 mM KCl, 87 μM NAD^+, 160 mM ethanol, 0.4 mg alcohol dehydrogenase, 0.4 mg purified catalase and EDTA particles[1] (1.2 mg protein). In the experiment of B the mixture contained 250 mM sucrose, 10 mM potassium succinate, 0.4 mg catalase and EDTA particles (1.5 mg protein). Respiration was started by adding 10 μl of 0.1% H_2O_2 to anaerobic particles. The pH traces refer to successive oxygen pulses made to the same particle suspension. The various additions were made sequentially in between the oxygen pulses. Temperature, 30°. A. Trace 1, before additions; Trace 2, after adding 3 μg oligomycin; Trace 3, after adding 0.5 μg valinomycin; Trace 4, after adding 0.5 μg nigericin. B. Trace 1, before additions, Trace 2, after adding 30 mM KCl; Trace 3, after adding 0.5 μg valinomycin; Trace 4, after adding 1 μg oligomycin; Trace 5, after adding 0.5 μg nigericin. The pH of the medium was measured with a highly sensitive rapidly responding pH meter. At equal H^+ gradients, the rate of H^+ leakage (on exhaustion of oxygen) in the presence of oligomycin was 30% (Expt. A) and 70% (Expt. B) lower than in the absence of oligomycin.

In Fig. 1B a similar experiment is shown in which potassium succinate was the substrate KCl (30 mM) gave a significant stimulation of the H^+ uptake, that was greatly potentiated by valinomycin. 1 μg Oligomycin (0.67 μg/mg protein) increased markedly the extent of the valinomycin-activated H^+ uptake and inhibited the rate of decay of the H^+ gradient on anaerobiosis. Nigericin greatly depressed the respiration-dependent proton gradient.

In Fig. 2 the effect of potassium salts, valinomycin and of various concentrations of oligomycin on the extent of the respiration-linked H^+ uptake is shown. The H^+ uptake was stimulated by both KCl and KNO_3, the latter being much more effective. Valinomycin was equally as effective as KNO_3 in stimulating H^+ uptake. Maximal stimulation was obtained when KCl or KNO_3 was added together with valinomycin. The oligomycin titration shows that, under all the conditions tested, 0.5–0.7 μg oligomycin per mg protein gave maximal stimulation of H^+ uptake. This value is equal to that obtained by LEE AND ERNSTER[1] in titrating the stimulatory effect of oligomycin on the energy-linked nicotinamide nucleotide transhydrogenase in the same type of particles.

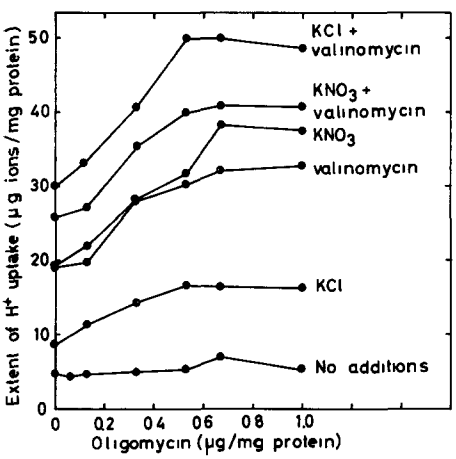

Fig. 2 Effect of potassium salts, valinomycin and oligomycin on the extent of respiration-linked proton uptake in submitochondrial particles. The experimental conditions were those described for Expt B of Fig. 1

Potassium salts stimulate the respiration-linked proton uptake in both Mg–ATP[11,13] and EDTA particles. The salts also uncouple oxidative phosphorylation, and there is a direct correlation between the degree of stimulation of H^+ uptake and that of uncoupling[13].

The uncoupling effect and the promotion of the respiration-linked uptake of H^+ by potassium salts are interpreted to be the consequence of an energy-expending cyclic transport of the cations. It is conceivable that potassium salts diffuse from the medium into the particles, that K^+ is then extruded by an energy expending process and that the latter is accompanied by H^+ uptake. The finding that KNO_3 is more effective than KCl in promoting H^+ uptake in the absence of valinomycin but that the difference disappears in the presence of this K^+-transporting antibiotic indicates that NO_3^- is a very effective counterion in supporting K^+ migration in the

membrane (see also ref. 3). The net rate of proton uptake is initially very fast. With succinate, in the presence of KCl and valinomycin, it can reach the value of 4 μg-ion H^+ per min per mg protein. The corresponding initial H^+/O ratio is 5 (see, however, ref. 14).

The net rate of H^+ uptake declines rapidly due to the depression of the inward flux and/or to enhancement of the rate of the passive back diffusion. The results presented show that oligomycin inhibits (see Fig. 1) the rate of the back diffusion of H^+ (see ref 14) and enhances the extent of the respiration-linked H^+ uptake. It is possible that as a consequence, the rate of the energy-dissipating cyclic transport of K^+ and that of respiration are slowed down. Such an inhibition would be released by nigericin, which exchanges[15] the H^+ taken up for K^+ from the medium (see ref. 8), or by uncouplers[1] due to their proton-conducting property[14].

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