SHORT COMMUNICATIONS

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The effect of monovalent cations on the dinitrophenol-induced ATPase of rat-liver mitochondria

Several years ago LARDY AND WELLMAN[1] showed that KCl and NaCl enhance the dinitrophenol-induced ATPase of rat-liver mitochondria, measured in a medium containing only neutralized ATP and sucrose, and this was confirmed by MYERS AND SLATER[2]. The nature of this stimulation has now been further examined.

With 6 mM ATP the dinitrophenol-induced ATPase was stimulated about 60 % when sucrose was replaced by an isomolar concentration of KCl or when KCl was added at a constant sucrose concentration (Fig. 1A). Varying the sucrose concentration had little effect (Fig. 1B). Thus the stimulation by KCl is not an osmotic effect. No stimulation by KCl was obtained in the presence of 10 mM $MgCl_2$.

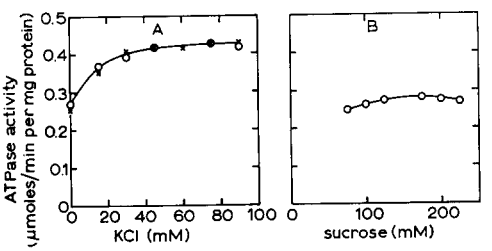


Fig. 1. Effect of KCl and sucrose concentrations on the dinitrophenol-induced ATPase of rat-liver mitochondria (prepared as in ref. 2). The reaction mixture contained 6 mM ATP (disodium salt brought to pH 7.5 with Tris), 0.1 mM 2,4-dinitrophenol, 0.5 mM EDTA (free acid brought to pH 7.5 with Tris), 0.18 mg/ml mitochondrial protein[10], and the concentrations of KCl and sucrose indicated. The reaction was started by adding the mitochondria. After 16 min at 25° the reaction was stopped with trichloroacetic acid to 5% and inorganic phosphate measured[11]. The values are corrected for P_i present in ATP and in mitochondria. A. ○, the osmolarity was kept practically constant by replacing sucrose by KCl, assuming 250 mM sucrose is isoosmolar with 150 mM KCl; ×, the sucrose concentration was kept at 75 mM. B. No added KCl.

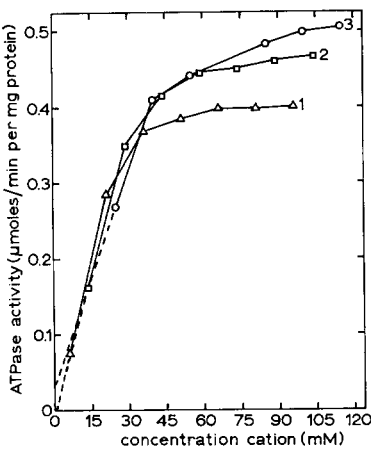
The maximum effect of KCl was obtained with about 50 mM. KCl could be replaced with NaCl (*cf.* ref. 1), LiCl, RbCl, CsCl, NH_4Cl, triethylammonium chloride and Tris chloride. KBr, KI and KNO_3 stimulated similarly, whereas KF was inhibitory above 6 mM (*cf.* refs. 1, 3). K_2SO_4 (and K_2SeO_4) stimulated much less than KCl, and inhibited in the presence of KCl. This inhibition was reversible by sedimenting the mitochondria by centrifugation and washing. The potassium salts of monocarboxylic acids (formate and acetate) and of dicarboxylic acids (oxalate, malonate, D-tartrate, L-tartrate, mesotartrate and maleate) stimulated to a smaller extent than KCl. Inhibition by anions of carboxylic acids, measured in the presence of KCl, have been reported by VELDSEMA-CURRIE AND SLATER[4].

In these experiments with 6 mM ATP, stimulation by added KCl or other salts

amounted to only about 60 %. However, even in the absence of added salts, approx. 24 mM Na^+ and $Tris^+$ are present to neutralize the ATP and about 1.5 mM to neutralize the EDTA. With lower concentrations of ATP, and consequently lower concentrations of cations, the stimulation by added cations was greater. Indeed when the ATPase activity is plotted against the total cation concentration, the lines extrapolated to zero cation concentration indicate an absolute requirement for cation for the dinitrophenol-induced ATPase, maximum activity being obtained with about 60 mM KCl (Fig. 2). This experiment shows clearly that it is the cation and not the anion that is responsible for the stimulation by added salts.

The requirement for a monovalent cation for the dinitrophenol-induced ATPase is rather unexpected, since monovalent ions (except NH_4^+) are only sluggishly permeable to the mitochondrial inner membrane, in the absence of an agent such as valinomycin[5]. It seems likely that the requirement is related to the difference in charge between ATP and ADP (*cf.* KLINGENBERG AND PFAFF[6] and see also ref. 7).

In all experiments described to date, a concentration of 0.1 mM 2,4-dinitrophenol was used. Fig. 3 shows the effect of varying both the KCl and the dinitrophenol concentration. The optimal concentration of dinitrophenol (*cf.* ref. 8) tends to increase with increasing KCl concentration.



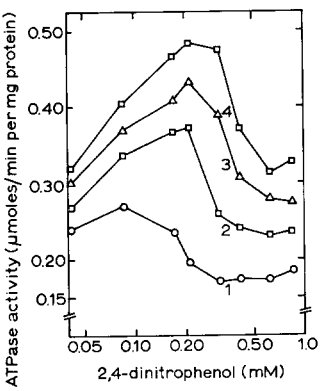


Fig. 2. Effect of added KCl on dinitrophenol-induced ATPase measured with different concentrations of ATP. The procedure was the same as in Fig. 1. 0.08 mg/ml mitochondrial protein, 0.1 mM 2,4-dinitrophenol, 0.5 mM EDTA. Reaction time, 12 min. On the abscissa the total concentration of monovalent cation is plotted. This is calculated as $4 \cdot [ATP] + 3 \cdot [KCl]$. The concentration of sucrose was 245, 237 and 225 mM with 1.2, 3 and 6 mM ATP, respectively, in the absence of added KCl, and the osmolarity was kept constant by replacing sucrose with KCl as in Fig. 1. Curve 1, 1.2 mM ATP; Curve 2, 3 mM ATP; Curve 3, 6 mM ATP.

Fig. 3. Effect of dinitrophenol concentration on ATPase activity measured in the presence of different concentrations of KCl. The procedure was the same as in Fig. 1. 0.14 mg/ml mitochondrial protein, 0.5 mM EDTA, 6 mM ATP. Reaction time, 15 min. Curve 1, 225 mM sucrose; Curve 2, 7.5 mM KCl, 212 mM sucrose; Curve 3, 15 mM KCl, 200 mM sucrose; Curve 4, 30 mM KCl, 175 mM sucrose.

R. KRAAIJENHOF (unpublished observation) has shown that the inhibition of the ATPase by high concentrations of dinitrophenol[8] is competitive with respect to the ATP concentration. These results and those depicted in Fig. 3 are understandable if

the inhibition by excess dinitrophenol is due to inhibition of the entry of ATP into the mitochondria (*cf.* ref. 9), and if K^+ promotes the entry of ATP, thereby allowing it to compete more effectively with the dinitrophenol.

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