THE STIMULATING ACTION OF K^+ ON THE HYDROLYTIC ACTIVITY OF SOLUBLE MITOCHONDRIAL ATPase

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SUMMARY

Potassium ions stimulate the hydrolytic activity of the soluble oligomycin-insensitive ATPase activity of bovine heart mitochondria. The stimulatory action of K^+ only occurs in the presence of an ATP generating system or in conditions in which the level of ADP is extremely low. A Lineweaver-Burk plot of ATPase activity measured at various concentrations of Mg-ATP shows that K^+ increases the Vmax and that it does not modify the Km for Mg-ATP. ATPase activity is not modified by Li^+ and Na^+, but Cs^+ induces a small stimulating action.

The reports on the effect of K^+ on oxidative phosphorylation are controversial. In K^+-depleted mitochondria, K^+ stimulates oxidative phosphorylation (1), but in submitochondrial particles K^+ diminishes the P:O ratios (2,3). In phosphorylating particles, K^+ diminishes the phosphorylation rate (3) and increases the rate of electron transport with NADH as substrate, but not with succinate (2,3). As in submitochondrial particles incubated in a non-phosphorylating system, it was found that K^+ increases the rate of the aerobic oxidation of NADH, but not that of succinate (4), it was concluded that K^+ is required for maximal rates of electron transport in the NADH-ubiquinone span. Nevertheless, it is also considered possible that the inhibiting action of K^+ on the phosphorylation reaction could be due to an action of the cation on the ATPase complex of the mitochondria. This is indeed a likely possibility since Adolfsen and Moudrianakis (5) showed that K^+ stimulated the ATPase activity of Alcaligenes faecalis and heart mitochondria.

In this work, we wish to describe experiments that indicate that K^+ stimulates the hydrolytic activity of the soluble oligomycin-insensitive ATPase (F_1) of heart mitochondria. This effect of K^+ seems to be controlled by the levels of ADP.

MATERIALS AND METHODS

Heart mitochondria were prepared according to Low and Vallin (6). Soluble oligomycin insensitive ATPase (F_1) was prepared according to a method that will be described elsewhere. The procedure consists of solubilization of F_1 by sonication of heart submitochondrial particles in the presence of ATP (7). The solubilized enzyme is adsorbed to a column of hexylammonium Sepharose (Pharmacia) and subsequently eluted with 1.0 M KCl, 0.25 M sucrose, 2 mM EDTA, and 2 mM ATP at pH 7.3. The eluted enzyme is precipitated with an equal volume of saturated $(NH_4)_2\ SO_4$ at pH 7.3. Precipitated F_1 was maintained as a half-saturated $(NH_4)_2\ SO_4$ solution until studies on its hydrolytic activity were conducted. Prior to the assay, the enzyme maintained in ammonium sulfate was centrifuged down and the precipitate dissolved in 0.25 M sucrose, 2 mM EDTA, and 2 mM ATP at pH 7.3.

F_1 thus prepared is approximately 80% pure on the basis of its specific activity (8) and electrophoretic pattern in sodium dodecyl sulfate gels. To measure ATPase activity, an aliquot of the solution (usually 5 µl containing 5 µg of protein) was added to the reaction mixture (detailed in the Results section). The reaction was stopped with 5% trichloroacetic acid final concentration and inorganic phosphate was determined according to Sumner (9). Protein was determined according to Lowry et al (10).

RESULTS AND DISCUSSION

Figure 1 shows the effect of K^+ on the ATPase activity of F_1 in the presence of an ATP generating system and in conditions in which ADP was not removed. In the former system K^+ induces a significant stimulation

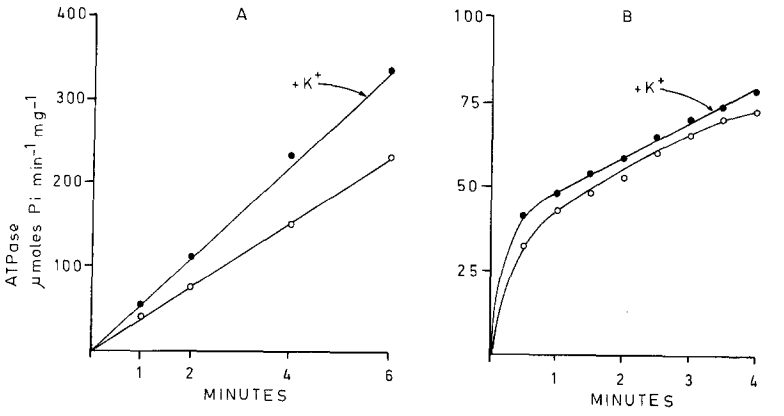

Figure 1. Effect of K^+ on the ATPase Activity of F_1. In A the incubating conditions were 10 mM tris-KCl pH 7.3, 4.0 mM Mg Cl_2, 5 mM phosphoenol-pyruvate, 10 μgrams pyruvate kinase (Sigma, 485 units per mg) ATP 2.2 mM, 20 mM KCl where shown and 10 μgrams of F_1. In B, the mixture contained 10 mM tris-HCl pH 7.3, ATP 2.2 mM, 4.0 mM $MgCl_2$, 20 mM KCl where shown and 10 μgrams F_1. In both experiments the temperature was 30°and the final volume was 1.0 ml.

of ATPase activity. In the absence of the ATP generating system, the overall activity is lower, and K^+ fails to stimulate the activity. This observation indicates that K^+ only stimulates ATPase activity in the absence of ADP.

In the presence of an ADP generating systems (hexokinase + glucose) Papa et al (2) and Christiansen et al (3) reported that K^+ diminishes the phosphorylation rate of submitochondrial particles. Also Papa et al (2) reported that K^+ competes with ADP in oxidative phosphorylation. With Mg-ATP as substrate, the data of Figure 2 show that K^+ increases the Vmax of the hydrolytic activity of F_1, whilst the Km for Mg-ATP is not modified by K^+. Therefore, the question is whether the action of K^+ on the hydrolytic activity of F_1 is related to its inhibiting action on the forward reaction (2,3).

Apparently this is not so, since the inhibiting action of K^+ on phosphorylation is reproduced almost exactly by Na^+ (2,3) while the effect of

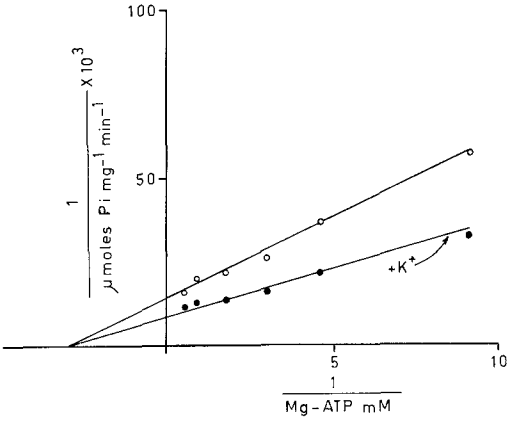

Figure 2. Effect of K^+ on the ATPase Activity of F_1 at various concentrations of Mg-ATP. The incubating conditions were 10 mM tris-HCl pH 7.3, the indicated concentrations of Mg-ATP, 5 mM phosphoenolpyruvate, 10 ugrams pyruvate kinase, Mg-ATP / Mg^{2+} free = 1, 10 μgrams F_1 and 20 mM KCl where shown. Incubation time, 5 min at 30°.

K^+ on the ATPase activity of F_1 is much more specific. Of the cations assayed, only K^+ and Rb^+ induced a strong activation of the reaction, while Na^+ and Li^+ hardly affected the activity (Table I); Cs^+ was less active than K^+ and Rb^+.

It is not clear whether the presently described stimulating action of K^+ on the hydrolytic activity of F_1 is related to its activating action on oxidative phosphorylation (1), but studies that will be published elsewhere indicate that K^+ stimulates the ATPase activity of submitochondrial particles and soluble F_1 in an almost identical form. Therefore the action of K^+ is not a consequence of the separation of F_1 from the membrane.

At the light of these findings, it is conceivable that the low phosphorylation activity of K^+-depleted mitochondria (1) may be the consequence of limiting ATPase activity due to lack of K^+. Moreover it is possible that in submitochondrial particles the stimulating action of K^+ on oxidative phosphorylation has not been detected because the experiments

Table I

Effect of Various Cations on the

ATPase Activity of F_1

Salt Added	ATPase Activity
	μmoles P_i mg^{-1} min^{-1}
-	30
L_1Cl	31
NaCl	33
KCl	44
RbCl	44
CsCl	36

The incubating conditions were as in Figure 1A, except that the incubation time was 5 min. The indicated salts were added at a concentration of 20 mM.

have been conducted in the presence of an ADP regenerating system (2,3,11) a condition in which the stimulating action of the cation is masked (see Fig. 1).

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