Journal of Bioenergetics and Biomembranes, Vol. 18, No. 2, 1986

On the Role of K^+ on Succinic Dehydrogenase Activity

Edmundo Chávez,[1,2] Concepción Bravo,[1] and David Jay[1]

Received March 4, 1985; revised August 22, 1985

Abstract

The effect of potassium ions on succinic dehydrogenase activity of mitochondria was studied. The results showed that in these organelles K^+ induces inhibition of the respiratory control; moreover, in submitochondrial particles potassium inhibits the rate of oxidation of succinate. The results showed also that K^+ does not changes the K_m for succinate but diminishes the V_{max}. In addition, the data provide evidence that mitochondria oxidizing glutamate-malate in a sucrose medium show a higher activity of succinate dehydrogenase than mitrochondria incubated in KCl.

Key Words: Mitochondria; succinic dehydrogenase; potassium; succinate; succinate oxidation; respiratory chain; heart succinic dehydrogenase; heart mitochondria.

Introduction

A number of studies have been carried out to understand the multiplicity of mechanisms that regulate the activity of mitochondrial succinic dehydrogenase. As a result of these studies, it is now well established that negative modulation of the enzyme can be induced by ADP, oxidized CoQ, and oxaloacetate; in contrast, ATP and reduced CoQ act as positive effectors of the enzyme (Singer *et al.*, 1973). In addition, it has been reported that monovalent anions such as formate, acetate, and bicarbonate induce reversible activation of the enzyme (Swierczynski and Davis, 1978). However, with the exception of an inhibitory effect of polyamines (Chaffee *et al.*, 1977), no effect of cations on succinic dehydrogenase has been described. This report

[1]Departamento de Bioquímica, Instituto Nacional de Cardiología, Ignacio Chávez, México, D.F. 014080, México.
[2]To whom correspondence should be addressed.

0145-479X/86/0400-0093$05.00/0

describes experimental results which show that potassium ions inhibit succinate oxidation.

Material and Methods

Mitochondria from dog and bovine hearts were prepared by the polytron technique as reported before (Jurkowitz *et al.*, 1974). Bovine heart mitochondria were used for the preparation of submitochondrial particles (SMP) according to the method described by Lee and Ernster (1966). Oxygen consumption was measured polarographically, with a Clark-type electrode. Succinate dehydrogenase activity was measured at 20°C by the phenazine methosulfate (PMS) and 2,6-dichlorophenolindophenol (DCIP) assay (Mowery *et al.*, 1977), in mixtures that are described in the Results section. Protein was determined by the procedure of Lowry *et al.* (1951).

Results and Discussion

Figure 1 shows the respiratory control of mitochondria oxidizing succinate in media that contain sucrose or KCl. It is observed that ADP added to mitochondria incubated in a sucrose medium induces a State 3/State 4 ratio of 2.7 (Fig. 1A); however, when the mixture contains KCl, the addition

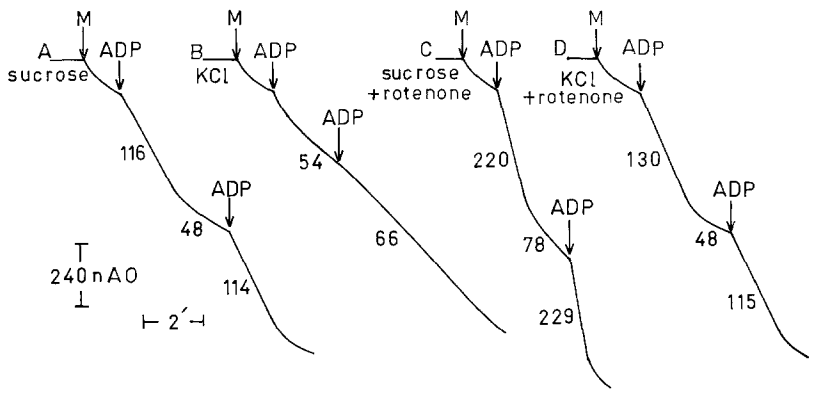

Fig. 1. The influence of KCl on the aerobic oxidation of succinate. Protein (2 mg) from mitochondria (M) were added to incubation mixtures containing 250 mM sucrose or 125 mM KCl; in addition the media contained 10 mM succinate-Tris, pH 7.3, 10 mM phosphate-Tris, ph 7.3, and, where indicated, 800 nmol ADP and 10 μg rotenone were added. The numbers indicate the respiratory rate as natoms oxygen per minute per milligram protein. Final volume 3 ml. Temperature 22°C.

Table I. Respiratory Rate of Mitochondria Supplemented with Cytochrome *c* in Sucrose and KCl Media

	natoms oxygen $min^{-1} mg^{-1}$		
Conditions	State 3	State 4	State 4 + cyt *c*
Sucrose	125	55	110
KCl	85	60	70

[a] mitochondrial protein (2 mg) was incubated under conditions described for Fig. 1 (traces A and B). As indicated, 5 μm cytochrome *c* was added.

of ADP to mitochondria induces a high initial State 3 respiratory rate that gradually diminishes to a State 4 respiratory rate (Fig. 1B). The latter observation would indicate an oxaloacetate type of inhibition of succinic dehydrogenase, which has been described by various authors (Ackrell *et al.*, 1974; Tuena *et al.*, 1969, 1972), and suggests that endogenously generated ATP is insufficient to prevent the binding of oxaloacetate to succinate dehydrogenase and subsequent inhibition of the enzyme is observed. The polarographic traces of Figs. 1C and 1D were obtained by the addition of mitochondria to sucrose or potassium chloride, respectively, that were supplemented with 10 μg rotenone in order to prevent oxaloacetate formation from malate.

In these conditions, without the influence of the negative modulator, the addition of ADP results in a comparable State 3–State 4 transition with parallel values of respiratory control, i.e., 2.9 and 2.8 for mitochondria respiring in sucrose or KCl medium, respectively (Fig. 1C and 1D). In addition, we would like to emphasize here the interesting and large difference that exists between the ADP-stimulated respiratory rate of mitochondria incubated in sucrose medium (Fig. 1C) versus that in KCl medium (Fig. 1D), 229 and 130 natoms oxygen $min^{-1} mg^{-1}$, respectively.

The experiments carried out with and without rotenone (Fig. 1) appear to indicate that KCl affects succinic dehydrogenase instead of another electron carrier. To test this assumption, cytochrome *c* was added and, as observed (Table I), cyt *c* is not able to stimulate oxygen consumption in a KCl medium, which is consistent with an inactivation of succinic dehydrogenase by K^+.

In addition, the results shown in Fig. 1 indicate a diminished State 4 respiratory rate in potassium medium in contrast to that observed in sucrose medium (Figs. 1C and 1D) This would be in apparent disagreement with the well-established observation that ATP acts as a positive effector of succinic dehydrogenase (Singer *et al.*, 1973), since after the addition of ADP, sufficient ATP would be present to stimulate the activity; however, it must be taken into account that in whole mitochondria adenine nucleotide translocase

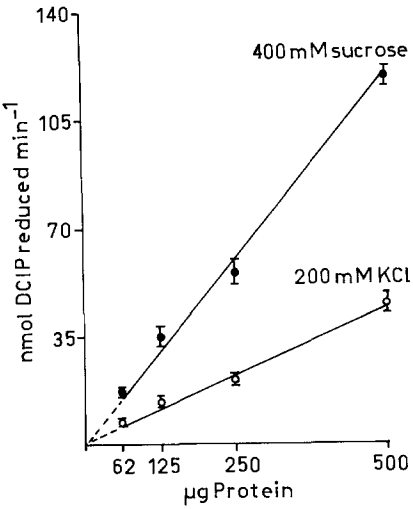

Fig. 2. Effect of potassium on succinic dehydrogenase activity at various levels of SMP. The activity was measured spectrophotometrically as described in Table II. The indicated amounts of protein (SMP) were incubated in the indicated concentrations of sucrose or KCl. The reaction was started by the addition of 10 mM succinate.

Table. II. Stimulation of Succinic Dehydrogenase Activity by ATP

Additions	nmol DCIP reduced $min^{-1} mg^{-1}$	
	KCl	Sucrose
–	78	185
3.6 mM ATP	153	300

[a] Protein (1 mg) from submitochondrial particles was preincubated 10 min in 1 ml of a medium which contained 10 mM Tris-HCl, pH 7.3, 10 µg oligomycin, and the indicated concentration of ATP. After the preincubation period, and aliquot of 0.1 ml (100 µg protein) was transferred to a medium containing 125 mM KCl or 250 mM sucrose in addition to 10 mM phosphate-Tris, pH 7.3, 10 mM Tris-HCl, pH 7.3, 1 mM EDTA, 1 mM NaCN, 0.5 mM phenazine methosulfate, and 50 µM DCIP for the analysis of the enzymatic activity. The reaction was started by the addition of 10 mM succinate.

removes ATP in exchange for ADP (Klingenberg and Pfaff, 1966), avoiding its accumulation and therefore its access to the locus of the enzyme; however, with the aim of clarifying whether ATP protects succinic dehydrogenase from potassium inactivation, an experiment was carried out in submitochondrial particles (SMP). The results of Table II show that preincubation of SMP with ATP (3.6 mM) results in an activation of the enzyme in both sucrose and KCl media.

The activity of membrane-bound succinic dehydrogenase in SMP was analysed at various protein concentrations in the presence of sucrose or KCl;

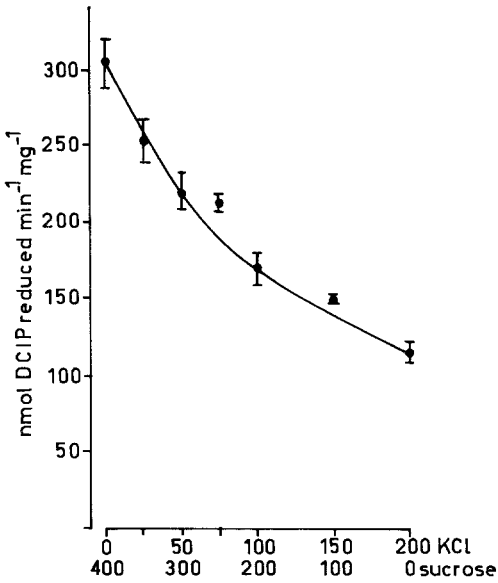

Fig. 3. Inhibition of succinate-DCIP reductase activity of SMP by increasing concentrations of KCl. Analysis of succinic dehydrogenase activity was carried out as described in Table II except for the indicated concentrations of sucrose and KCl that were added to the incubation medium.

it is observed that a 60% inhibition of succinate dehydrogenase by potassium occurs at all the concentrations of protein utilized (Fig. 2).

To ascertain the concentration of KCl that induces 50% inactivation of succinic dehydrogenase, the experiment shown in Fig. 3 was performed; the concentration of KCl was increased and the tonicity of the medium was fixed by varying the concentration of sucrose. The K_i value was 147 mM, a concentration which is within the physiological levels of potassium inside heart mitochondria (Tedeschi, 1981).

In regard to the different rates of respiration in sucrose media, the data of Fig. 4 show that potassium produces a decrease in the V_{max} for succinate, which would suggest that potassium induces a conformational alteration of the enzyme to an inactive form.

It has been reported by Gutman *et al.* (1971) that there is a decline in succinate dehydrogenase activity when ADP is added to mitochondria oxidizing α-ketoglutarate as substrate; such inactivation appears to be be due to an increase in the CoQ_{ox}/CoQ_{red} ratio. We have investigated similarly whether succinic dehydrogenase becomes more inhibited when mitochondria consuming malate-glutamate as substrates are incubated in KCl than in sucrose medium; the experiment of Fig. 5 demonstrates agreement with this assumption.

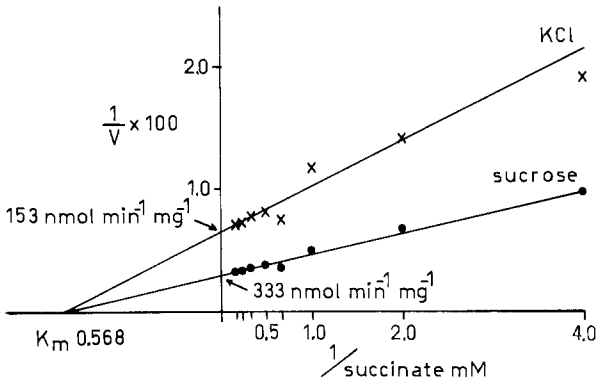

Fig. 4 Double reciprocal plot for the effect of KCl on succinic dehydrogenase activity measured at different concentrations of succinate. The activity of succinic dehydrogenase was assayed as in Table II. Succinate concentrations were varied as indicated.

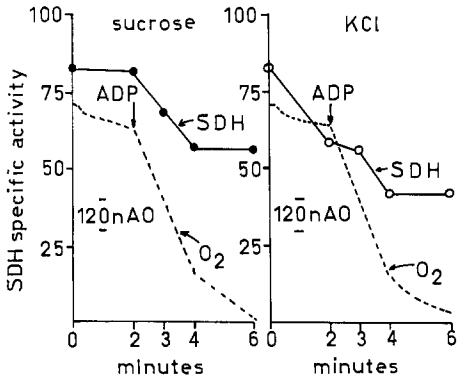

Fig. 5 Different inactivation of succinic dehydrogenase (SDH) in State 3 and State 4 in sucrose and potassium media. Mitochondrial protein (2 mg) was incubated in a medium containing 250 mM sucrose or 125 mM KCl; in addition the media (3 ml) contained 10 mM malate and 10 mM glutamate adjusted to pH 7.3 with Tris, and 10 mM phosphate-tris. Where indicated, 800 nmol ADP was added. At the indicated times, aliquots of 100 μl (66 μg protein) were transferred from the oxygraph to a medium containing 10 mM phosphate-Tris, pH 7.3, 10 nM Tris-HCl, pH 7.3, 1 mM EDTA, 1 mM NaCN, 0.5 mM phenazine methosulphate, and 50 μM DCIP, and the reaction was immediately started by the addition of 10 mM succinate for the spectrophotometric analysis of succinic dehydrogenase.

The KCl-induced inhibition of succinic dehydrogenase can be reproduced by other salts such as naCl, choline chloride, or $NaNO_3$ (not shown). However, the inhibitory effect of potassium must be considered important since this is the most abundant cation inside mitochondria (Tedeschi, 1981); moreover, it has been reported by Gómez-Poyou *et al.* (1972) that K^+ plays an important role as a positive effector of oxidative phosphorylation at

Site I. Therefore, if potassium stimulates oxidation of NAD-dependent substrates, it would be conceivable that the cation inactivates succinic dehydrogenase with the purpose of avoiding mutual interference in the oxidation of each other by exceeding maximum capacity of the respiratory chain for electron transport; however, it is worthwhile to recognize that this regulatory role proposed for potassium on succinate oxidation is difficult to sustain since, as shown (Fig. 5), the inactivation is not reversible. From this latter point of view potassium-induced inhibition would be the result of a stable conformational modification of the enzyme in such a way that it becomes more sensitive to the negative modulators such as oxaloacetate or oxidized CoQ.

References

Ackrell, B. A. C., Kearny, E. B., and Mayr, M. (1974). *J. Biol. Chem.* **249**, 2021–2027.

Chaffee, R. R. J., Salganicoff, L., Arim, R. M., Rochell, R. H., and Schltz, E. L. (1977). *Biochem. Biophys. Res. Commun.* **77**, 1009–1016.

Gómez-Puyou, A., Tuena, M., Sandoval, F., Chávez, E., and Peña, A. (1972). *Biochemistry*, **11**, 97–102.

Gutman, M., Kearney, E. B., and Singer, T. P. (1971). *Biochemistry*, **10**, 4763–4770.

Jurkovitz, M., Scott, K. M., Altschuld, R., Merola, A. J., and Brierley, G. P. (1974). *Arch. Biochem. Biophys.* **165**, 98–113.

Klingenberg, M., and Pfaff, E. (1966). In Symposium on the Regulation of Metabolic Processes in Mitochondria (Tager, J. M., Papa, S., Quagliariello, E., and Slater, E. C., eds.), Elsevier, North-Holland, New York–Amsterdam, Vol. 7, pp. 180–201.

Lee, C. P., and Ernster, L. (1966). In Symposium on the Regulation of Metabolic Processes in Mitochondria (Tager, J. M., Papa, S., Quagliariello, E., and Slater, E. C., eds.), Elsevier, North-Holland, New York–Amsterdam, Vol. 7, pp. 218–234.

Lowry, O. H., Rosenbrough, N. J., Farr, A. L., and Randall, R. (1951). *J. Biol. Chem.* **193**, 265–275.

Mowery, P. C., Steekamp, D. J., Ackrell, B. A. C., Singer, T. P. and White, G. A. (1977). *Arch. Biochem. Biophys.* **178**, 495–506.

Singer, T. P., Kearney, E. B., and Kenney, W. C. (1973) *Adv. Enzymol* **37**, 189–272.

Swierczynski, J., and Davis, J., (1978). *Biochem. Biophys. Res. Commun.* **85**, 1173–1179.

Tedeschi, H. (1981). *Biochim. Biophys. Acta* **639**, 157–196.

Tuena, M., Gómez-Puyou, A., Peña, A., Chávez, E., and Sandoval, F. (1969). *Eur. J. Biochem.* **11**, 283–290.

Tuena de Gómez-Puyou, M., Chávez, E., Freites, D., and Gómez-Puyou, A. (1972). *FEBS Lett.* **22**, 57–60.