THE EFFECT OF SUCCINATE, MALONATE AND FUMARATE ON THE PHOSPHORYLATING SYSTEM OF THE SUBMITOCHONDRIAL PARTICLES

V.V. KUPRIYANOV and V.A. SAKS
Laboratory of Bioorganic Chemistry, Moscow State University, Moscow, USSR

Received 10 April 1972

1. Introduction

It is known that succinate dehydrogenase of submitochondrial particles can be activated by succinate, by its competitive inhibitors and also by reduced CoQ_{10} [1–3] or by NADP(P)H [4] its conformation being thereby changed [1, 2]. Recently Gutman et al. [5] have shown that the activity of mitochondrial succinate dehydrogenase may be regulated not only by the above agents, but also by ATP. It was previously shown that ATP changes the kinetic properties and thermal lability of succinate dehydrogenase from rat brain mitochondria [6]. One of the explanations of this effect was that ATP is capable of changing the succinate dehydrogenase environment, thereby affecting its structure [6].

It has been shown in the present investigation that the agents (succinate, malonate, fumarate), which are known to specifically interact with succinate dehydrogenase can cause essential changes in the phosphorylating system of submitochondrial particles. Hence, it is possible to suggest that the conformational changes induced in succinate dehydrogenase by its activators, can in some way affect the structure of its immediate surroundings and the properties of other enzymic systems of submitochondrial particles.

2. Materials and methods

Submitochondrial particles $ETP_H(Mg^{2+}, Mn^{2+})$ were isolated from heavy mitochondria of beef heart as described by Beyer [7]. The P/O ratio, the rate of the ATP-^{32}P exchange, ATPase activity and the rate of the ATP-dependent reduction of NAD^+ by succinate was measured as described previously [8]. When the ATP-dependent reduction of NAD^+ by TMPD with ascorbate was measured the medium contained, instead of succinate, 0.25 mM TMPD and 0.25 mM ascorbate. When the particles were preincubated with succinate, the measurement of the rate of ATP-^{32}P exchange, ATPase activity and the rate of ATP-dependent reduction of NAD^+ by TMPD with ascorbate was made in the medium containing 0.3 μmoles tenoyl trifluoracetone per mg protein or 0.8 mM malonate. When the particles were preincubated with trypsin the assay medium contained soya trypsin inhibitor (5 mg per mg trypsin).

The particles (3.0 mg protein per ml) were incubated at 30° in the medium containing 2 mM $MgCl_2$, 10 mM potassium phosphate, pH 7.5, 5 mM Tris-HCl, pH 7.5, 0.5 mM EDTA, 33 mM glucose and 100 mM sucrose. For details see the figure captions.

3. Results and discussion

As is seen in fig. 1, preincubation of submitochondrial particles with succinate in anaerobic conditions results in an increase of the maximal rate of the ATP-dependent reduction of NAD^+ by succinate and in the disappearance of the lag-phase in this reaction (fig. 1A). This effect is not due to the increased rate of electron transfer because of succinate dehydrogenase activation, as was previously suggested by Gutman et al. [4]. In fact, the preincubation of the particles in the conditions indicated above was accompanied by an increased in the rate of the ATP-dependent reduction of NAD^+ by TMPD with ascorbate (fig. 1B), although the latter reaction involves no succinate dehy-

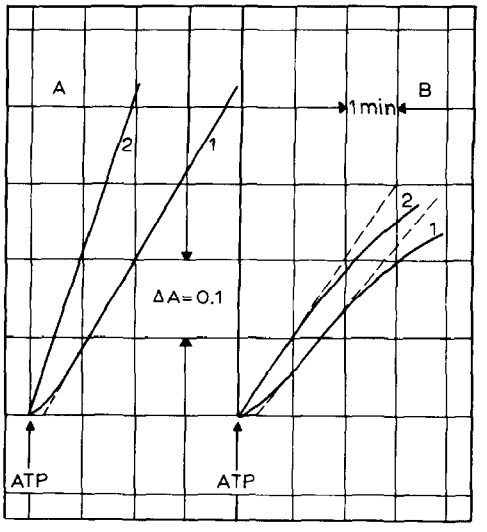

Fig. 1. The effect of the preincubation of submitochondrial particles with succinate in anaerobic conditions on the ATP-dependent reduction of NAD^+ by A) succinate and B) TMPD with ascorbate. 1: Untreated particles. 2: The particles incubated with succinate. Submitochondrial particles at a concentration of 3.0 mg/ml were incubated in anaerobic conditions with 7.0 mM succinate for 6 min at 30° in the medium described in Materials and methods. A concentration of the submitochondrial particles in the spectrophotometer cuvette was 0.15 mg/ml. Temperature was 30°.

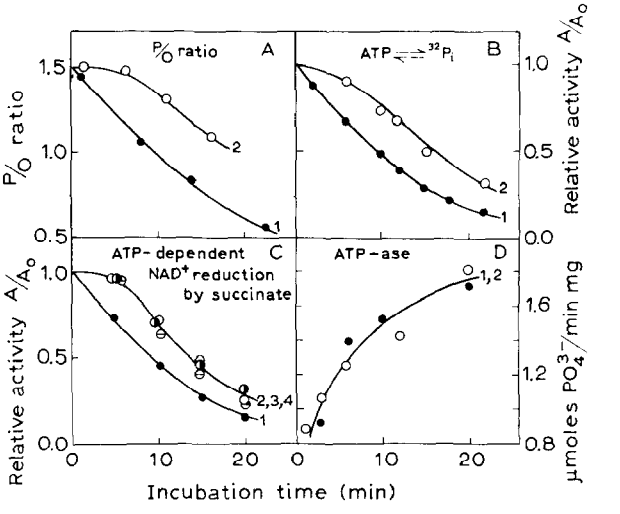

Fig. 2. The effect of succinate, malonate and fumarate on proteolysis of the phosphorylating system of submitochondrial particles. A) The change in the P/O ratio (succinate as substrate) on the incubation of the particles with trypsin (18 μg/ml). 1: Control. 2: Succinate (7 mM), anaerobic conditions. B) The change in the rate of the ATP-^{32}P exchange reaction. 1: Control (A_0 = 59 nmoles ATP^{32} per mg protein per min). 2: Succinate (7 mM), anaerobic conditions (A_0 = 69). C) The change in the rate of NAD^+ reduction by succinate in the presence of ATP. 1) Control (A_0 = 119 nmoles NADH per mg protein per min). 2) Succinate (7 mM) anaerobic conditions (A_0 = 193). 3) Malonate (1 mM), aerobic conditions (A_0 = 119). 4) Fumarate (20 mM) aerobic conditions (A_0 = 119). A_0 was measured after a 6 min preincubation of the particles with succinate, malonate or fumarate. D) The change of the ATPase activity. 1) Control. 2) Succinate (7 mM), anaerobic conditions.

drogenase. Moreover, after preincunation of the particles with succinate the rate of the ATP-^{32}P exchange reaction increased by 20–30%. This means that the results presented in fig. 1 testify to the phosphorylating system being activated by succinate in anaerobic conditions. This effect did not occur in the presence of oxygen.

The action of succinate in anaerobic conditions is not only that of activating the phosphorylating system. Fig. 2 shows that succinate in anaerobic conditions protects the phosphorylating system from proteolysis which is revealed in the retardation of the decrease in the P/O ratio, the rate of the ATP-dependent reduction of NAD^+ by succinate as well as in the rate of ATP-^{32}P exchange on treatment of the particles with trypsin. This effect should be interpreted to mean that in the absence of oxygen*

* In aerobic conditions succinate does not increase the resistance of the phosphorylating system to trypsin [8].

succinate in some way induces specific structural changes in the phosphorylating system. It is noteworthy that succinate does not affect trypsin-induced activation of ATPase (fig. 2D).

Besides succinate, malonate and fumarate also produce a stabilizing effect on the phosphorylating system in anaerobic conditions (fig. 2A).

The question arises why succinate, malonate and fumarate which are known to specifically interact with succinate dehydrogenase, produce a marked effect on the phosphorylating system of SMP. It has been shown that these compounds activate succinate dehydrogenase by inducing in it conformational changes, the succinate activation being especially

pronounced in anaerobic conditions [1–3, 9]. Since nothing is known about the direct interaction of dicarboxylic acids with the phosphorylating system it remains to suggest that the structural changes in succinate dehydrogenase are somehow extended to the phosphorylating system, which results in the increased resistance of the latter to trypsin. For example, it would be possible in the case when succinate dehydrogenase and Factor F_3 which is known to possess high sensitivity to trypsin [10] where localized in close proximity to each other in the cristae membrane. Such a suggestion holeds if one has in mind the model of the inner mitochondrial membrane suggested by Racker [11] and Sjöstrand [12], according to which succinate dehydrogenase is closely related to some components of the phosphorylating system. An alternative approach to such local structural changes is a cooperative rearrangement of the whole membrane resulting from the altered structure of succinate dehydrogenase.

References

[1] E.B. Kearney, J. Biol. Chem. 229 (1957) 363.
[2] T. Kimura, J. Hauber and T.P. Singer, J. Biol. Chem. 242 (1967) 4987.
[3] M. Gutman, E.B. Kearney and T.P. Singer, Biochemistry 10 (1971) 2726.
[4] U.F. Rasmussen, FEBS Letters 19 (1971) 239.
[5] M. Gutman, E.B. Kearney and T.P. Singer, Biochem. Biophys. Res. Commun. 44 (1971) 526.
[6] M. Tuena, A. Gomex-Puyou, A. Pena, E. Chavex and F. Sandoval, European J. Biochem. 11 (1969) 283.
[7] R.E. Beyer, in: R. Estabrook and M. Pullman, Methods in Enzymology, Vol. 10 (Acad. Press, New York, 1967) p. 186.
[8] V.N. Luzikov, V.A. Saks and V.V. Kupriyanov, Biochem. Biophys. Acta 253 (1971) 46.
[9] V.A. Saks, L.V. Romashina and V.N. Luzikov, Biokhimiya 35 (1970) 797.
[10] E. Racker, Proc. Natl. Acad. Sci. U.S. 48 (1962) 1659.
[11] E. Racker, Essays in Biochem. 6 (1970) 1.
[12] F.S. Sjöstrand and L. Barajos, J. Ultrastructure Res. 32 (1970) 293.