INHIBITION OF SUCCINATE OXIDATION AND TRANSPORT INTO MITOCHONDRIA BY METAL-COMPLEXING AGENTS

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1. Introduction

Studies of the permeability properties of mitochondria indicate that the cristae membrane contains several systems used for the transport of substrates into and from the matrix space, including a dicarboxylic acid transporter system [1, 2]. The discovery of some inhibitors of these systems [3–7] has enabled progress to be made in understanding their chemical nature and the reactions that they catalyse [2].

This communication describes the inhibition of succinate oxidation by some metal-complexing agents and presents evidence that their site of action is situated in the dicarboxylic acid transporter system. The results suggest that a metal ion may be an essential component of this transporter system.

2. Materials and methods

Sodium bathophenanthroline sulphonate and tannic acid were obtained from the Sigma Chemical Company. Butylmalonate (Aldrich Chemical Company) was a gift from Dr. R.B.Beechy. Other reagents were obtained from Hopkin and Williams Ltd.

Rat liver mitochondria were isolated in 0.25 M sucrose by the method of Schneider [8]. Respiration rates were measured by the polarographic technique with a Clark oxygen electrode [9]. The reaction mixture (volume 3.2 ml) contained 0.25 M sucrose, 15 mM KCl, 5 mM $MgCl_2$, 8 mM K phosphate buffer and 2 μg of rotenone, at pH 7.4 and 25°, together with mitochondria (4.5 mg of protein) and various concentrations of K succinate and inhibitor as indicated. Respiration was stimulated by the addition of either 0.6 mM ADP or 0.1 mM 2,4-dinitrophenol (DNP).

The extinction at 750 nm due to light-scattering of mitochondrial suspension was measured in a dual-wavelength scanning spectrophotometer (Phoenix Precision Instruments, Philadelphia, Pa., U.S.A.). A decrease in extinction was assumed to be due to mitochondrial swelling. The reaction mixture (volume 3.0 ml) contained 80 mM ammonium succinate, 5 mM tris-HCl buffer, 0.1 mM EGTA, 4 μg of antimycin and 4 μg of rotenone, at pH 7.4 and 25°, together with mitochondria (3.0 mg of protein) and various concentrations of inhibitor as indicated. Swelling was stimulated by the addition of 3 mM ammonium phosphate.

3. Results and discussion

The addition of bathophenanthroline sulphonate to a suspension of liver mitochondria oxidizing succinate, in the presence of either ADP and phosphate or 2,4-dinitrophenol, caused an immediate inhibition of respiration (fig. 1). Since bathophenanthroline was an equally effective inhibitor of both phosphorylating and uncoupled respiration, the inhibition appeared to be situated at the respiratory-chain level, and not in the phosphorylation system. The inhibition of uncoupled succinate oxidation by a fixed concentration of inhibitor was decreased when the succinate concentration was increased, suggesting that bathophenanthroline was acting as a competitive inhibitor. This conclusion was confirmed by measurements of the effect of bathophenanthroline concentration on the

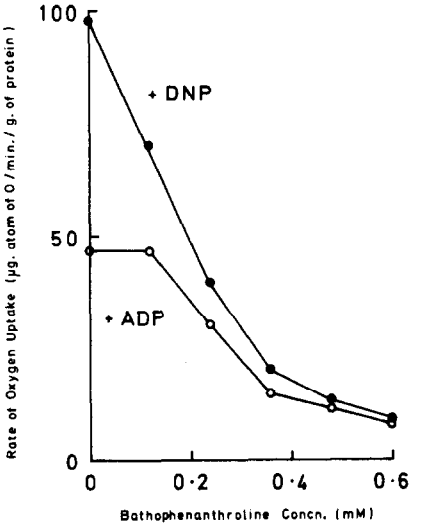

Fig. 1. Effect of bathophenanthroline sulphonate concentration on the rate of succinate oxidation stimulated by ADP or DNP. The succinate concentration was 5 mM.

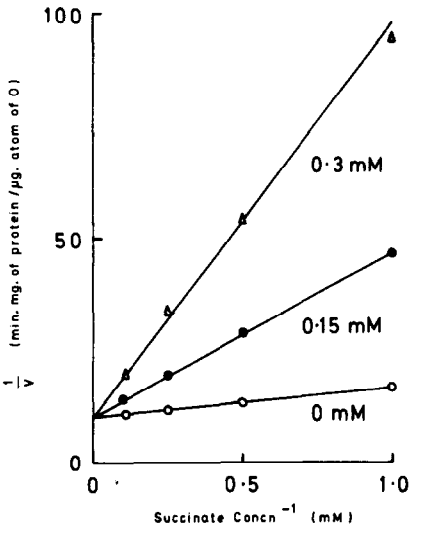

Fig. 2. Double reciprocal plots of the rate of succinate oxidation (DNP-stimulated) against succinate concentration in the presence of various concentrations of bathophenanthroline sulphonate as indicated.

rate of succinate oxidation at various substrate concentrations. The data obtained are presented in the form of double reciprocal plots (fig. 2). The K_m for succinate in the absence of inhibitor (0.48 mM) was increased to 9 mM in the presence of 0.3 mM bathophenanthroline, but no change was observed in the value of V_{max}. Palmer [10] concluded that the inhibition of coupled succinate oxidation by 5 mM bathophenanthroline sulphonate was due to an action on the phosphorylation system and not at the respiratory-chain level. In his interesting experiments, the competitive inhibition of succinate oxidation by bathophenanthroline sulphonate was probably overcome by the high concentration of succinate (100 mM) that he used, thus enabling an inhibition of the phosphorylation system to be observed.

The inhibition of uncoupled succinate oxidation by bathophenanthroline (fig. 1) was also decreased by the addition of cytochrome *c* (0.2 μmoles/g of protein) with a membrane-lytic concentration of Triton X-100 (0.02%, v/v). Bathophenanthroline sulphonate had no significant effect on succinate oxidation when the mitochondrial suspension was sonicated before assay. These observations suggested that the action of the inhibitor was on the entry of succinate via the dicarboxylic acid transporter, and not on the succinate oxidation system. Succinate transport into mitochondria was therefore investigated by studying the swelling of mitochondria in solutions of ammonium succinate containing a low concentration of ammonium phosphate [1]. Mitochondrial swelling in ammonium succinate (or ammonium malate) was inhibited by bathophenanthroline sulphonate (fig. 3), thus confirming that this compound is an inhibitor of the dicarboxylic acid transporter.

The inhibition of succinate oxidation by bathophenanthroline was compared with that induced by two other inhibitors of the dicarboxylic acid transporter, namely butylmalonate [3] and tannic acid [7]. The data obtained (fig. 4) are presented in the form of Dixon plots [11]. The plot was linear when butylmalonate was the inhibitor used, in agreement with the conclusion [3] that this reagent is a true competitive inhibitor. In contrast, the plots obtained with tannic acid and bathophenanthroline sulphonate were non-linear, indicating that these reagents are partially competitive inhibitors [12] and do not act directly at the succinate binding site.

We have found that other metal-complexing agents,

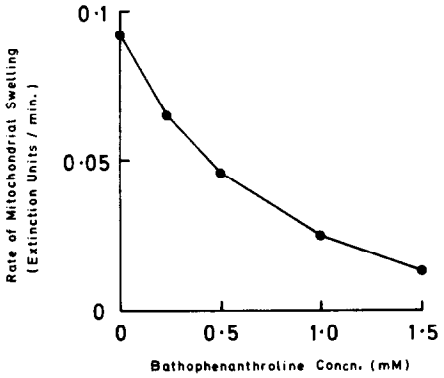

Fig. 3. Effect of bathophenanthroline sulphonate concentration on the initial rate of phosphate-induced swelling of mitochondria suspended in ammonium succinate.

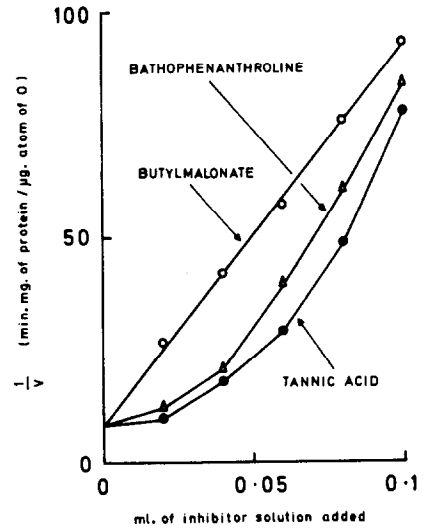

Fig. 4. Dixon plots of the inhibition of succinate oxidation. The succinate concentration was 5 mM. The concentrations of inhibitor solutions used were: butylmalonate, 0.5 M; bathophenanthroline sulphonate, 20 mM; tannic acid, 2.5 mM. Aliquots of these inhibitor solutions were added to the reaction mixture as indicated.

including bathocuproine, *o*-phenanthroline, 8-hydroxyquinoline and tiron (catechol disulphonic acid) also act as competitive inhibitors of uncoupled succinate oxidation. The fact that several chemically distinct metal-complexing reagents exert a similar inhibitory action suggests that their effects are due to their common metal-complexing activity. Some of the reagents have strong affinities for ferrous ions, but they also form complexes with a number of other metals [13]. It is therefore postulated that the reagents form a complex with a metal ion, possibly non-haem iron, which is involved in the mechanism of anion exchanges catalysed by the dicarboxylic acid transporter system.

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