RECONSTITUTION OF RESPIRATORY CONTROL OF SUCCINATE OXIDATION IN SUBMITOCHONDRIAL PARTICLES

Chuan-pu Lee and Birgitta Johansson

Johnson Research Foundation, University of Pennsylvania
Philadelphia, Pa. 19104

and

Tsoo E. King

Department of Chemistry, SUNY, Albany, N.Y. 12203

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SUMMARY

Reconstitution of respiratory control and the energy-dependent pyridine nucleotide transhydrogenation linked to succinate oxidation by soluble succinate dehydrogenase and the dehydrogenase-depleted particles is demonstrated. The restoration of succinate oxidation and respiratory control is a function of the concentration of succinate dehydrogenase present. Externally added succinate dehydrogenase effects neither the rate nor the respiratory control of NADH oxidation. The results are discussed in terms of the structural organization of the respiratory components in the mitochondrial membrane.

Respiratory control was first demonstrated by Lardy and Wellman (1) and then elaborated by Chance and Williams (2). It is one of nature's most efficient self-regulating and self-adjusting mechanisms for sequential reactions in the multi-enzyme systems in mitochondria. For some years it was generally accepted that respiratory control is an intrinsic property of intact mitochondria and could not be demonstrated in submitochondrial particles. Indeed, one of the great surprises of the work on reconstruction of oxidative phosphorylation in the laboratories of Green (3) and Racker (4,5) was their failure to achieve respiratory control under conditions that resulted in oxidative phosphorylation. Recent work by Lee and Ernster (6-8) shows that respiratory control can be induced in submitochondrial particles by treatment with oligomycin, and three to four-fold changes in the rate of electron flow have been observed when the oligomycin supplemented system is treated with an uncoupling agent. Kinetic studies (9) of the respiratory chain carriers indicated that oligomycin exhibits a multi-site control of electron transport in submitochondrial particles,

similar to that found with intact mitochondria (2). The present paper communicates the results on reconstitution of respiratory control and a related energy manifested reaction linked to succinate oxidation in submitochondrial particles by soluble succinate dehydrogenase and the dehydrogenase-depleted particles.

MATERIALS AND METHODS

EDTA particles derived from "heavy" beef heart mitochondria were prepared as described previously (10). The succinate dehydrogenase in EDTA particles was removed by treating the particles at pH 9.5 for 30 minutes at 37° C (alkali-treated particles), essentially the same as previously used for removal of succinate dehydrogenase from Keilin-Hartree preparation (11). Soluble succinate dehydrogenase was prepared from beef heart as described by King (11). Oxygen consumption was measured polarographically with a Clark oxygen electrode. The energy-linked pyridine nucleotide transhydrogenation was assayed according to Ernster and Lee (12). Carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP) was a gift of Dr. P.G. Heytler, E.I. du Pont de Nemours and Co., Wilmington, Delaware.

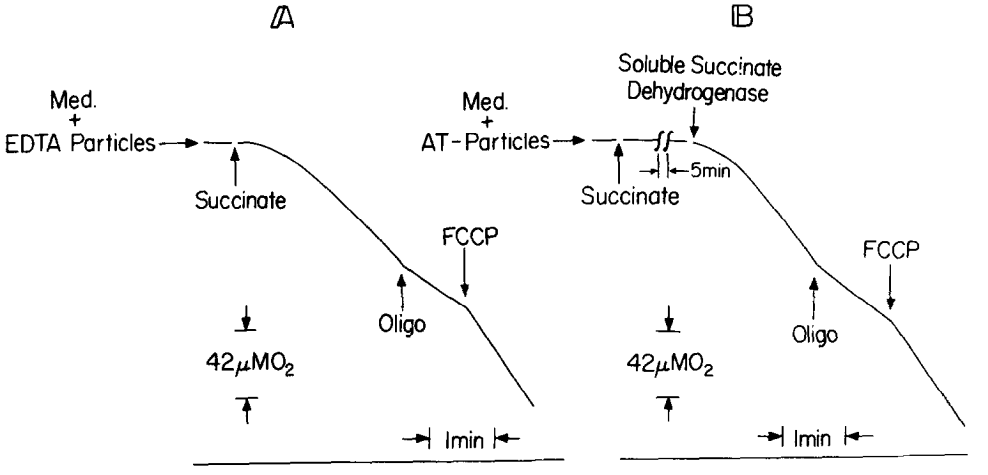

Figure 1. Restoration of respiration and respiratory control of succinate oxidation by soluble succinate dehydrogenase

The reaction mixture consisted of 180 mM sucrose, 50 mM Tris-acetate buffer, pH 7.5, and submitochondrial particles (in A, 0.6 mg protein of EDTA particles; in B, 0.5 mg protein of the alkali-treated particles, AT-particles). 5 mM succinate, 3 µg oligomycin, 1 µM FCCP, and 12 µg protein of soluble succinate dehydrogenase were added when indicated. Final volume, 3 ml; temperature, 30° C.

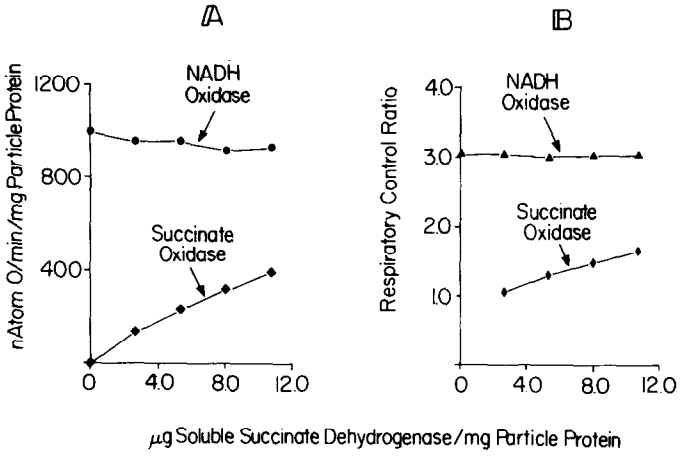

Figure 2: Effect of varying amounts of soluble succinate dehydrogenase on the rate (A) and respiratory control (B) of both NADH oxidase and succinate oxidase activities of the alkali-treated particles

Varying amounts of soluble succinate dehydrogenase were mixed before assays with the alkali-treated particles at ratios indicated in the Figure. The assay conditions were the same as that described in Figure 1A except that in the assay of NADH oxidase, 0.3 mg of particle protein was present and 1 mM NADH was used as substrate. The respiratory control ratio was expressed as the ratio of the respiratory rates after and before the addition of FCCP to the oligomycin supplemented system.

RESULTS AND DISCUSSION

EDTA particles showed a respiratory control ratio of about 1.7 with succinate as substrate (Figure 1A). The capacity in succinate oxidation was completely abolished after alkali treatment (Figure 1B). Addition of soluble succinate dehydrogenase to the system restored not only the oxidation, but also respiratory control of the alkali-treated particles to the original value. The restoration of succinate oxidation and respiratory control was dependent upon the amount of succinate dehydrogenase present. This type of titration is depicted in Figure 2. Alkali treatment of EDTA particles does not alter either the rate or the respiratory control of NADH oxidation (cf. ref. 7,8). Furthermore, as shown in Figure 2 the presence of externally added succinate dehydrogenase effects neither the rate nor the respiratory control of NADH oxidation.

The question arises as to whether the soluable succinate dehydrogenase is physically re-incorporated into the particles or whether

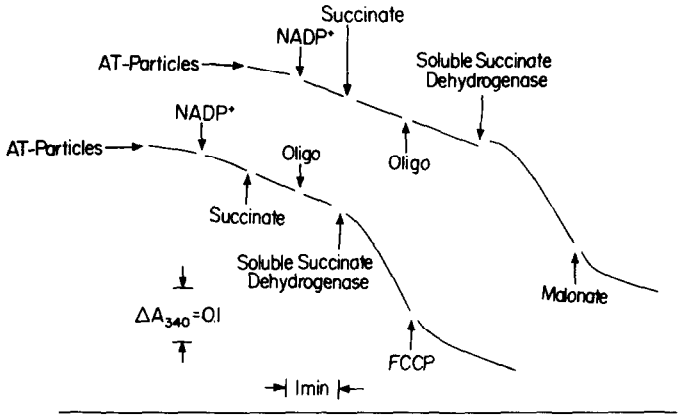

Figure 3: Restoration of the energy-linked pyridine nucleotide transhydrogenation by soluble succinate dehydrogenase

The reaction mixture consisted of 180 mM sucrose, 50 mM Tris-acetate buffer, pH 7.5, 3.3 μM rotenone, 0.2 mM NADH, 0.66 mM oxidized glutathione and an amount of glutathione reductase capable of oxidizing 0.5 μmoles of NADPH/min, and 1 mg protein of alkali-treated particles. 0.2 mM $NADP^+$, 5 mM succinate, 3 μg oligomycin, 12 μg protein of soluble succinate dehydrogenase, 1 mM malonate, 1 μM FCCP were added when indicated. Final volume, 3 ml; temperature, 25°C.

the reaction is merely due to some sort of random collision. A mixture of excess succinate dehydrogenase and alkali-treated particles, similar to that used in Figure 2, was centrifuged and the pellet was washed. The particles thus re-isolated showed the same oxidative and control capacities as the mixture did; the excess succinate dehydrogenase remained in the supernatant (cf. ref. 13).

Since the reactions involving succinate oxidation and respiratory control have been reconstituted, it is almost imperative to examine the manifestation of other energy-linked reactions. We studied the energy-linked reduction of $NADP^+$ by NADH with oxidized glutathione and glutathione reductase as the $NADP^+$ generating system. As is clearly shown in Figure 3, the activity of this reaction is restored by the addition of soluble succinate dehydrogenase. This reaction is sensitive to both malonate and FCCP.

A number of salient points may be deduced from these observations. It appears that the alkali-treated EDTA particles have all the structural and functional requirements for electron transfer and energy coupling except succinate dehydrogenase. In other words, the alkali treatment only removes succinate dehydrogenase from the particles without affecting other activities. Once the dehydrogenase is

re-incorporated into the particles, the complete re-assembled multi-enzyme apparatus can again perform the same functions as the original. In recent years certain advances have been made, especially by Green (3) and Racker (4,5), on the reconstruction of oxidative phosphorylating systems utilizing various coupling factors. However, their systems may lack some structural elements since only phosphorylation, but not respiratory control, is partially restored with or without the aid of oligomycin.

Studies of proton translocation (14,15) linked to the oxidation of succinate or NADH, and ATP hydrolysis in submitochondrial particles; as well as the binding properties (16-19) of cytochrome c in intact mitochondria and submitochondrial particles, support the idea that sonication of mitochondria causes fragmentation and "pinching off" of the cristae. This fragmentation gives rise to vesicles whose outer surface corresponds to the inner surface of the cristae as first proposed by Lee and Ernster (7). From the results described in this note and those to be reported elsewhere, we are inclined to believe that succinate dehydrogenase is localized on the outer surface of the submitochondrial vesicles (i.e., the inner surface of the mitochondrial cristae) as witnessed by the ease of the dissociation and reconstitution of the dehydrogenase without impairment of other activities. Recently we have shown (16,19) that cytochrome c can be incorporated into the inner surface of submitochondrial vesicles; only then does it show all properties of "endogenous" cytochrome c. This fact may explain the failure to remove cytochrome c from the Keilin Hartree preparation (20), the success in its removal of at least 90% of the endogenous cytochrome c from mitochondria (21), and the greater accessibility of succinate to succinate dehydrogenase in Keilin Hartree preparation than intact mitochondria (22). With succinate dehydrogenase located on the outer surface of the submitochondrial vesicles (i.e., the inner surface of mitochondrial cristae), cytochrome c and cytochrome oxidase (16-19) on the inner surface of the submitochondrial vesicles, further localization of the remaining respiratory chain carriers in the membrane may determine the role of structural organization of the chain in relation to the mechanism and the efficiency of energy coupling in intracellular respiration.

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