ACTIVATION OF NADH OXIDASE BY SUCCINATE IN PARTIALLY UBIQUINONE-DEPLETED SUBMITOCHONDRIAL PARTICLES

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1. Introduction

The point of convergence of the succinate and NADH branches of the respiratory chain appears to be important in controlling electron flow from the primary dehydrogenases [1]. This control point is extremely complex, containing ubiquinone (UQ), non-heme iron [1,2] and perhaps an unidentified lipid factor [3]. It has been stated [1], however, that UQ alone is the common connection between the dehydrogenases and the cytochrome chain. This suggestion is based primarily on the demonstration that partial removal, or incorporation, of UQ limits the activity of both NADH and succinate oxidase. Furthermore, it would appear from such studies [4–7] that UQ is rate limiting for both chains.

In the present paper we present evidence for the existence of an additional component which can, under certain conditions, become rate limiting to both NADH and succinate oxidase. This component is controlled by the activational state of succinate dehydrogenase, but does not appear to be part of the molecule per se.

2. Materials and methods

Submitochondrial particles were derived from heavy beef-heart mitochondria by sonication in the presence of EDTA [8]. UQ-depleted particles were prepared by extracting lyophilized particles 4 times with dry pentane [4]. Known amounts of UQ were incorporated into the UQ-depleted particles by the addition of UQ in pentane, followed by a slow and gradual removal of the pentane under a slight reduced pressure in a rotary evaporator [5]. The incorporation was carried out at 4°C and after complete removal of the pentane the particles were dried in a vacuum evaporator.

The reaction mixture consisted of 167 mM sucrose and 50 mM Tris–acetate, pH 7.5. All reactions were carried out at 30°C and at a final volume of 3 ml. NADH oxidase and succinate oxidase activities were measured with a Clark oxygen electrode in the presence of 0.33 mg particle protein/ml and 1 mM NADH, respectively 5 mM succinate. NADH-ferricyanide reductase activity was measured as described earlier [7] except that 0.83 mM malonate was present. Succinate dehydrogenase (SDH) activity was measured as described [9], except that a fixed (0.3 mM) phenazine methosulfate concentration was used. NADH-cytochrome *c* reductase and succinate-cytochrome *c* reductase activities were measured spectrophotometrically at 550 nm with 1 mM NADH and 0.83 mM malonate or 5 mM succinate, respectively, in the presence of 0.33 mg cytochrome *c*/ml and 1.7 mM KCN. NADH-duroquinone reductase activity was recorded at 340 nm in the presence of 0.2 mM NADH, 0.83 mM malonate, 0.2 mM duroquinone, and 1.7 mM KCN.

Unless otherwise indicated, activation of SDH with succinate was carried out at 10 mg/ml protein concentration in the presence of 20 mM succinate for 30 min at 30°C.

3. Results

In agreement with earlier reports [5], partial re-incorporation of UQ (2.5 nmoles/mg protein) into

Table 1
Effects of pretreatment with succinate on the respiratory chains of submitochondrial particles with different UQ-content

Enzyme reaction	Lyophilized particles			Extracted particles			Partially UQ-replenished particles		
	Control	Succinate pretreated particles	% activation	Control	Succinate pretreated particles	% activation	Control	Succinate pretreated particles	% activation
	(nmoles·min^{-1}·mg^{-1})			(nmoles·min^{-1}·mg^{-1})			(nmoles·min^{-1}·mg^{-1})		
Succinate oxidase	450	440	0	38	48	48	215	450	110
Succinate-cytochrome *c* reductase	485	570	18	53	56	5	202	475	135
NADH oxidase	490	480	0	19	19	0	180	370	106
NADH-cytochrome *c* reductase	455	485	0	18	18	0	130	255	97
NADH-duroquinone reductase	261	250	0	45	45	0	121	143	17
NADH-ferricyanide reductase	18.6	18.6	0	18.8	18.8	0	18.4	18.4	0

Experimental conditions as described in Materials and methods. The NADH-ferricyanide reductase activity is expressed in μmoles·min^{-1}·mg^{-1}. The extracted and partially UQ-replenished particles contained 0.3 and 2.5 nmoles UQ, respectively.

UQ-depleted submitochondrial particles leads to partial reactivation of both succinate oxidase and NADH oxidase activities. Reactivation of the respiratory activities follows saturation kinetics as a function of UQ concentration [4,5], suggesting that UQ is rate limiting unless present at its normal concentration (6 nmoles/mg protein [4]). Table 1 shows, however, that preincubation of the particles with succinate not only activates SDH [9–11], but also succinate oxidase and NADH oxidase. Activation of the latter activities is observed only in particles containing part of its normal UQ content, but not in lyophilized or fully UQ-replenished preparations. The succinate-induced activation of *both* succinate and NADH oxidase is significant since it indicates that: (i) under these conditions UQ is probably not the rate limiting component; (ii) the rate limiting component, though activated by succinate, participates in the electron transfer process in *both* branches of the respiratory chain.

Table 1 also shows the localization of the activated site along the respiratory chain, as determined by various electron acceptors. NADH oxidase and NADH-cytochrome *c* reductase were activated approximately 100%, whereas NADH-duroquinone reductase and NADH-ferricyanide reductase were not significantly affected. Since duroquinone accepts electrons from the oxygen side of UQ ([7–12] table 1), the lack of activation of NADH-duroquinone reductase must indicate that UQ itself is not the activated site. Thus it appears that an increase in oxidation of NADH is related either to (i) a component associated with the SDH molecule, whose participation in the NADH oxidase can be promoted by incubation with succinate, or (ii) to a component which is activated by reduction *via* SDH. To test this, the particles were incubated under different conditions. The results (table 2) indicate that malonate, though an activator or SDH [10], does not stimulate NADH oxidase. Furthermore, when succinate incubation is carried out in the presence of TTFA, no enhancement of NADH oxidase is observed. The control value (table 2) was not inhibited by TTFA since the latter was diluted during the assay. These data suggest that activation of NADH oxidase is not associated with the dehydrogenase but does, however, require reduction by SDH. On the other hand, NADH can not activate the component (table 2), indicating a close relationship between the activated site and SDH.

That the activated component is not a part of SDH is also suggested by the data in fig. 1 showing a different time scale for the increase in SDH activity

Table 2
Effects of incubation conditions on succinate and NADH oxidase activities in partially UQ-incorporated submitochondrial particles

Incubation conditions	Succinate oxidase	NADH oxidase
	(nmoles $0 \cdot min^{-1} \cdot mg^{-1}$)	
Control	167	167
20 mM Succinate (30 min)	308	294
20 mM Succinate + 100 mM TTFA (30 min)	119	119
2 mM Malonate (7 min)	164	164
0.25 mM NADH (5 min)	120	190
0.25 mM NADH (10 min)	119	182)

Experimental conditions as described in Materials and methods and as indicated in the table. The particles contained 2.5 nmoles UQ/mg protein.

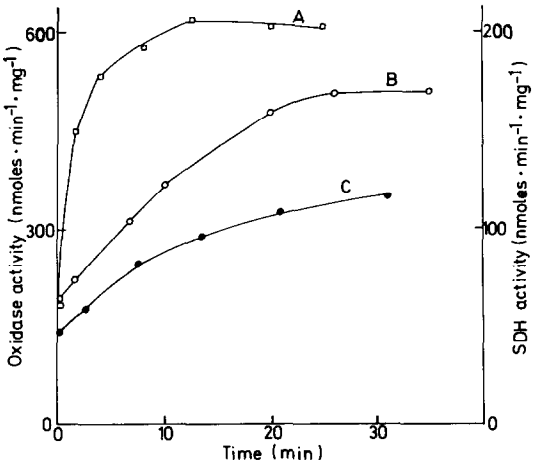

Fig. 1. Activation of SDH, succinate oxidase and NADH oxidase by succinate. Experimental conditions as described in Materials and methods: A) (□–□–□) SDH activity; B) (○–○–○) NADH oxidase activity; C) (●–●–●) Succinate oxidase activity. The particles contained 2.5 nmoles UQ/mg protein.

and the two oxidase activities upon incubation with succinate. The slow time-dependent activation also indicates that the increase in NADH oxidase is not an artifact of the measuring system.

4. Discussion

The present study shows the existence of a component of the respiratory chain which participates in the oxidation of both succinate and NADH, and whose function is controlled by the activational state of SDH. Visualization of the participation of this component is dependent upon the UQ content of the particles. When UQ is rate limiting (particles with partial UQ content) preincubation with succinate activates *both* succinate oxidase and NADH oxidase, but no effect is obtained when the particles contain its full complement of UQ. It has generally been considered that oxidation or reduction of UQ is rate limiting to respiration since saturation kinetics are obtained by its partial removal [4], or by re-incorporation back to UQ-particles [5–7]. The finding that respiratory chain is limited by 50% of its maximal activity upon partial removal of UQ (table 1), but can still be activated by incubation with succinate, suggest the existence of a rate limiting step other than UQ. The component involved appears to be influenced by a rather complex set of conditions, including the activated state of SDH and the concentration of UQ.

In spite of the fact that activation of the component is related to the activation of SDH, considerable evidence indicates that it is not part of the SDH molecule *per se*. Its activation, rather, seems to require reduction via SDH. This is shown by the findings that neither malonate, an activator of SDH [10], nor succinate in the presence of TTFA activates NADH oxidase in partially UQ-depleted particles. Both malonate and TTFA [2] lead to oxidation of part of the SDH. Additional evidence suggesting that the component in question is not part of SDH is: (i) activation of SDH by succinate is more rapid than activation of either succinate oxidase or NADH oxidase, and (ii) the activated site appears to be localized on the oxygen side of SDH. The latter is shown by our findings that succinate pretreatment activates NADH-cytochrome *c* reductase and NADH oxidase but not NADH-duroquinone reductase. Duroquinone accepts electrons on the oxygen side of UQ (ref. [12], table 1 of this paper) interacting either with a non-heme iron protein [13] or directly with cytochrome *b* [14]. Thus it seems reasonable that the succinate-activated component described in this report is located in the same segment of the chain.

Activation of NADH oxidase is best considered as a tool to visualize the component in question, rather than a normal physiological function of the component. The finding that the unidentified component becomes rate limiting to NADH oxidase only when the membrane is partially UQ-depleted suggests a close relationship between it and UQ. A high concentration of UQ apparently prevents the component from becoming rate limiting. The nature of the component is not known but resembles in many respects the non-heme iron protein of Crane and Hare [13] or the component through which UQ is thought to regulate the reduction of cytochrome *b* by SDH [4,15,16].

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