RECONSTITUTION OF RESPIRATORY CHAIN ENZYME SYSTEMS. X. RECONSTITUTION OF SUCCINATE OXIDASE WITH CYTOCHROME c-CYTOCHROME OXIDASE OF HEART MUSCLE

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The preceding communication has presented evidence for the formation of a complex with a 1:1 ratio of cytochrome c to cytochrome oxidase. Other forms of the complex with ratios of 0.2 and 0.5 have also been described. The variation in composition is apparently due to the polymerization state of the cytochrome oxidase employed in the reaction mixture. Among these forms, the complex of ratio 1 is most active in the reconstitution of succinate oxidase with soluble succinate dehydrogenase and the cytochrome b-c_1 particle.

Materials and Methods

The methods of preparation of soluble succinate dehydrogenase, the cytochrome b-c_1 particle and the reconstituted succinate cytochrome c reductase were described previously (Takemori and King, 1962; King and Takemori, 1962). Oxygen consumption was measured by polarography with a GME Oxygraph Model K.

Results and Discussion

Soluble succinate dehydrogenase, the cytochrome b-c_1 particle, the reconstituted succinate cytochrome c reductase or any form of cytochrome c-cytochrome oxidase complex alone, was inactive in the catalytic oxidation of succinate by molecular oxygen. However, in the presence of either soluble succinate dehydrogenase plus cytochrome b-c_1 particle or the reconstituted succinate cytochrome c reductase, the cytochrome c-cytochrome oxidase complex of 1 to 1 ratio actively mediated the transfer of hydrogen (electron) from succinate to oxygen. In this system, free cytochrome c was not required. Figure 1 shows the time course of the oxygen consumption of the reconstituted

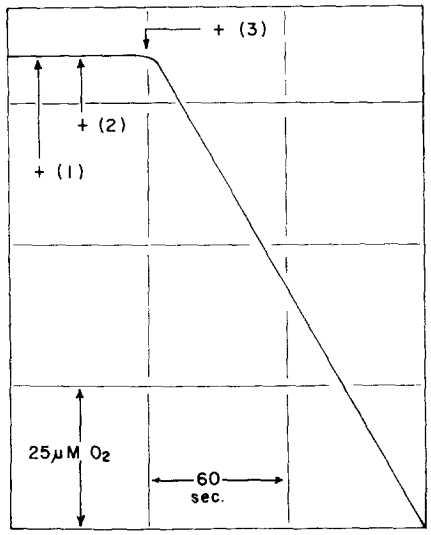

Figure 1. The reconstitution of succinate oxidase with succinate cytochrome c reductase and the cytochrome c-cytochrome oxidase complex.

The reaction mixture contained the final concentrations of 25 mM phosphate buffer, 40 mM succinate, succinate cytochrome c reductase and the cytochrome c-cytochrome oxidase complex; total volume, 2.5 ml; pH 7.4; temperature, 25°. The reductase consisted of 80 μg of soluble succinate dehydrogenase at the gel eluate stage and 1.2 mg of cytochrome b-c_1 particle per ml. The cytochrome c and the cytochrome oxidase contents of the complex were 1.2 μM each. Additions: (1) succinate; (2) succinate cytochrome c reductase and (3) the cytochrome c-cytochrome oxidase complex.

system. The catalytic activity of the system was proportional to the concentration of the cytochrome c-cytochrome oxidase complex. When a constant amount of succinate cytochrome c reductase was titrated with the complex, the activity measured as oxygen consumption increased linearly at first and gradually fell off as shown in Figure 2. It can be noted that the Q_{O_2} value of approximately 55,000 μl of oxygen consumed per hour per μmole of cytochrome oxidase content of the complex was obtained for the reconstituted succinate oxidase at 25°. The activity of the system was almost completely inhibited by the usual respiratory poisons, such as Antimycin A, cyanide, and azide, but not by Amytal. It was also inhibited by thenoyltrifluoroacetone and competitively by malonate.

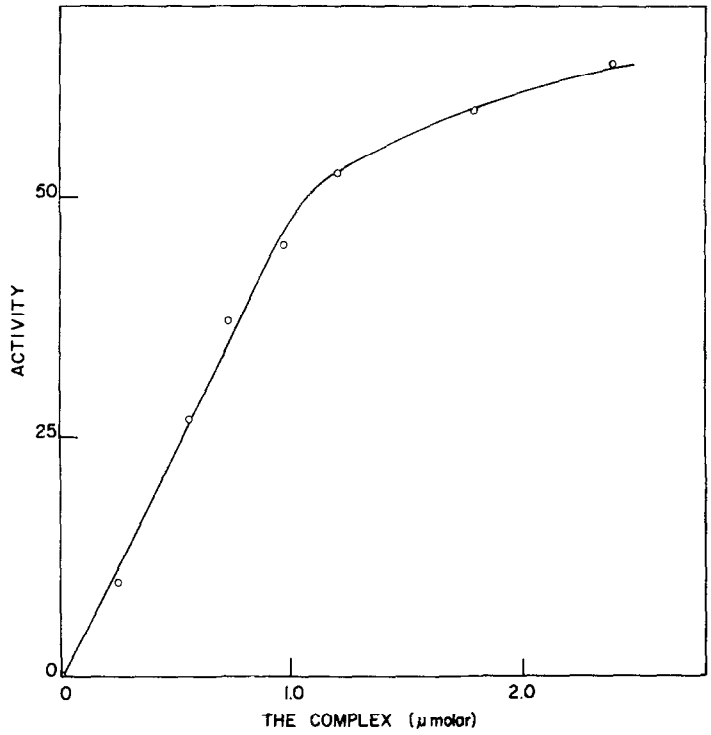

Figure 2. The effect of the concentration of the cytochrome c-cytochrome oxidase complex on the activity of the reconstituted succinate oxidase system.

The reaction mixture contained 25 mM phosphate buffer, 40 mM succinate, succinate cytochrome c reductase (80 μg of soluble succinate dehydrogenase plus 1.2 mg of the cytochrome b-c_1 particle in 1 ml) and indicated amounts of the cytochrome c-cytochrome oxidase complex; total volume, 2.5 ml; pH 7.4. The ordinate is the activity in μM of oxygen consumed at about 25° per minute. The abscissa is the concentration of the complex (1:1 molar ratio of cytochrome c to cytochrome oxidase) in μM cytochrome oxidase present.

Table I shows the reconstitutive activity of the complex of different ratios of cytochrome c to cytochrome oxidase. It can be seen that the activity increases with the ratio. Furthermore, in the reconstitution of succinate oxidase, the specific activity of the complex of 1:1 ratio of cytochrome c to cytochrome oxidase was nearly twice as much as that of its components when the latter were added in the free form (cf. Table I). On the other hand, those forms of the complex with the lower ratios of cytochrome c to cytochrome oxidase exhibited almost the same activity as if the components

were in the un-combined state. The lower activity of the systems with free components comparable to the complex was not due to the highly polymerized state of the cytochrome oxidase used, since the latter had been sonically treated immediately before the assay. Mention should also be made that the behavior of the complex in the catalytic oxidation of ascorbate was the same as in the reconstitution of succinate oxidase.

Table 1. Comparison of the activity of the cytochrome c- cytochrome oxidase complex with the free components in the reconstitution of succinate oxidase

Molar ratio of the complex ($\frac{\text{cytochrome c}}{\text{cytochrome oxidase}}$)	Specific activity (μl O_2 consumed/minute/ μmole cytochrome oxidase)		Activity ratio ($\frac{\text{complex}}{\text{free components}}$)
	Complex	Free Components	
1.0	760	420	1.8
.5	340	280	1.2
.2	190	170	1.1

The reaction mixture contained 25 mM phosphate buffer, 40 mM succinate, succinate cytochrome c reductase (70 μg of succinate dehydrogenase plus 1.3 mg of the cytochrome b-c_1 particle) and the complex or "free components" at 1.0 μM of cytochrome oxidase; total volume, 2.5 ml; pH 7.4; temperature 25°. "Free components" (cytochrome c and cytochrome oxidase) were added in the same ratio as that in the complex. Before addition, the cytochrome oxidase was subjected to sonic oscillation for about 45 minutes.

These results, coupled with other considerations which will be elaborated elsewhere, would suggest that the complex of ratio 1:1 (cytochrome c to cytochrome oxidase) is not an artifact and may exist as such in the intact respiratory chain. Nevertheless, more experiments are needed to firmly establish its physiological significance in the electron transfer systems of heart muscle.

Acknowledgment

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References

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