RECONSTITUTION OF SUCCINATE-Q REDUCTASE

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Received October 31,1977

SUMMARY--Reconstitution of succinate-Q reductase is achieved by admixing soluble succinate dehydrogenase (SDH) and ubiquinone-protein-S (QP-S), a new protein isolated from the soluble cytochrome $\underline{b}$-$\underline{c}_1$ complex. The reconstituted reductase catalyzes reduction of Q by succinate. The reaction is fully sensitive to thenoyltrifluoroacetone. The reconstituted reductase (same as succinate-cytochrome $\underline{c}$ reductase or submitochondrial particles) does not show "low concentration ferricyanide reductase activity" as soluble dehydrogenase does. In other words, this enzymic site on SDH is occupied by QP-S. When an artificial dye, such as phenazine methosulfate or Wurster's Blue, is used as electron acceptor the rate of oxidation of succinate by SDH is not significantly changed regardless of whether the dehydrogenase is in the free or in the reconstituted succinate-Q reductase forms.

Freshly prepared soluble, reconstitutively (1) active succinate dehydrogenase possesses two distinct ferricyanide reductase activities. One requires low ferricyanide concentrations ($K_m \sim 50$ μM), while the other needs high concentrations ($K_m \sim 10$ mM) (2, 3). It has been claimed that a correlation exists among the following three parameters: reconstitutivity, low-ferricyanide activity (4) and the appearance of the HiPIP type (high potential iron protein as shown by EPR technique) iron sulfide center ($\underline{i.e.}$ Center S-3) (5). In addition, succinate dehydrogenase can also use phenazine dyes (6, 7) and Wurster's Blue (8), but not ubiquinone (Q)[1], or 2,4-dichlorophenolindophenol (DCIP) (1) among others, as artificial electron acceptors. In regard to the action of inhibitors, thenoyltrifluoroacetone (TTFA) barely inhibits the artificial activities of the soluble enzyme but markedly (in micromolar quantities) inhibits succinate oxidation catalyzed by reconstituted or intact succinate-cytochrome $\underline{c}$ reductase as well as succinate oxidase (see Fig. 1 of

[1] Abbreviations used: DCIP, dichlorophenolindophenol; HiPIP, high potential iron protein; PMS, phenazine methosulfate; Q, ubiquinone; QP-S, ubiquinone-binding protein in the succinate dehydrogenase region; SDH, succinate dehydrogenase; TTFA, thenoyltrifluoroacetone; and WB, Wurster's Blue.

ISSN 0006-291X

Ref. 1). It is also well documented that the soluble enzyme is unstable with half-lives of about 0.5 and 8 hours, respectively, for reconstitutive and artificial activities at 0-4° in the presence of air (1).

Recently we have demonstrated the existence and isolation of a ubiquinone binding protein (QP-S, previously called QP-1) which can serve as an electron (hydrogen) carrier from succinate to ubiquinone (9). This paper reports reconstitution of succinate-Q reductase from QP-S and SDH. In addition, the locus of TTFA inhibition and other properties delineating the differences between free succinate dehydrogenase and dehydrogenase bound to QP-S, or as it exists in the respiratory chain, are also reported.

MATERIALS AND METHODS

Succinate dehydrogenase (1, 7), the cytochrome $\underline{b}$-$\underline{c}_1$ complex (10), the heart muscle preparation (11), and Wurster's Blue (12) were prepared by previously reported methods. QP-S[2] was prepared as briefly described in (9); details will be published elsewhere. The concentrations of electron acceptors were measured spectrophotometrically and the millimolar extinction coefficients (7, 12) used were 5.2 at 510 nm, 21 at 600 nm, and 1.0 at 410 nm, for Wurster's Blue, DCIP, and ferricyanide, respectively. Enzymic assays were conducted spectrophotometrically at room temperature, about 23°. The reaction mixture, in a final volume of 1 ml, contains 50 mM Na-K phosphate buffer, pH 7.8; 1.5 mM KCN; 1 mg/ml bovine serum albumin; 40 mM succinate and various amounts of proper electron acceptors (cf. Ref. 7). It must be mentioned that cyanide was added by following the conventional method (see Ref. 13 for easier comparison of data appearing in earlier literature), rather than for the possible contamination of cytochrome oxidase. Indeed, all QP-S and SDH described in this paper are completely free of any cytochrome. Spectrophotometric measurements were done in a Cary spectrophotometer, model 16, with a recorder.

RESULTS AND DISCUSSION

Figure 1A shows the actual tracing of the low-ferricyanide reductase activity of freshly prepared SDH. The assay was conducted with 350 μM $K_3Fe(CN)_6$ at 23°. The specific activity, as calculated by the initial slope of the tracing, is 13 μmoles[3] succinate oxidized per min per mg protein. When this preparation was simply admixed with excess amounts of QP-S (i.e. about 5 mol QP-S per mol of SDH) and then assayed under the same conditions

[2] A part of Q of the QP-S isolated has dissociated in the course of isolation and purification.

[3] The range of activity is usually between 12 and 16 μmoles of succinate oxidized per min per mg of SDH at room temperature.

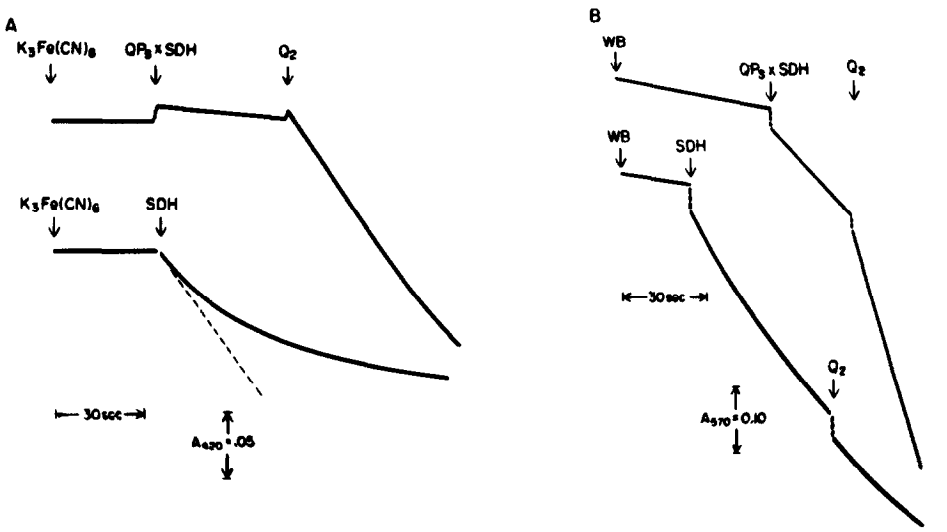

Fig. 1. Activities of succinate-dehydrogenase and reconstituted succinate-Q reductase. (A) Ferricyanide and (B) Wurster's Blue were used as electron acceptors: 5 µl of freshly prepared SDH, 0.77 mg/ml, were used in the assay. In the reconstituted system, excess QP-S (5-fold of the dehydrogenase) was mixed with SDH; the concentration of SDH was also adjusted to 0.77 mg/ml.

practically no activity (< 5%) of free SDH was observed in the absence of exogenous Q (Fig. 1A); this indicates that SDH is now being converted to succinate-Q reductase and the active site of the so-called low ferricyanide reductase activity of SDH is occupied. Formation of succinate-Q reductase is further evidenced by the fact that upon the addition of Q, the reduction of ferricyanide was stimulated to the same level as that in the initial rate of free SDH. Also, unlike the free enzyme, reconstituted succinate-Q reductase activity does not decay with time; whereas free SDH inactivates rapidly in air (cf. Ref. 1). The slower rate shown at the end of the tracing was due to the approach of exhaustion of ferricyanide in the reaction mixture. The reaction of QP-S toward (i.e. QP-S bound to) SDH is identical to that of the cytochrome b-c_1 complex (10) or alkaline treated particles functionally (cf. Ref. 14). The cytochrome b-c_1 complex or alkaline treated particles not only reconstitute with SDH to form succinate-cytochrome c re-

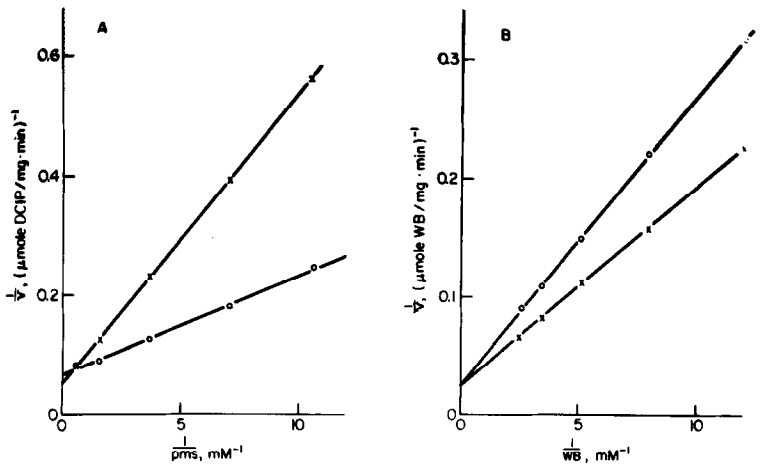

Fig. 2. Double reciprocal plots for SDH and reconstituted succinate-Q reductase activity against acceptor concentrations. The conditions for the enzymes used were identical to those detailed in Fig. 1. The concentrations of the immediate acceptors of PMS (A) and Wurster's Blue (B) in the reaction mixture are given in the plots. The concentration of DCIP in (A) was kept at constant, 45 mM. In all the PMS systems described in this paper, the final acceptor was DCIP.

ductase or succinate oxidase, respectively, but they also abolish the low ferricyanide reductase activity of SDH at the same time.

When WB (260 μM) was used as the electron acceptor, similar results, as shown in Fig. 1B, were observed. In this case the results were complicated by the fact that both free SDH and reconstituted succinate-Q reductase are able to catalyze the reduction of WB (cf. Ref. 15). The stimulation of this dye reduction by exogenous Q also confirms the formation of succinate-Q reductase; whereas the rate of reduction of WB catalyzed by SDH in the presence of succinate is not affected by the addition of Q.

Figure 2 depicts double reciprocal plots of free SDH and reconstituted succinate-Q reductase activities toward WB and PMS assay systems. When WB was used as acceptor it resulted in a slight decrease in K_m for the dye but not the $V_{max}^{succinate}$. In the PMS system significant increase both in K_m^{PMS} and $V_{max}^{succinate}$ was observed in reconstituted succinate-Q reductase. It should be

TABLE I. K_m and V_{max} of Succinate Dehydrogenase and Reconstituted Succinate-Q Reductase.

Preparations	Electron Acceptors					
	Succ. → PMS-DCIP		Succ. → Q-DCIP		Succ. → WB	
	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}
Succinate dehydrogenase	0.3	18	-	-	1.1	41.7
Succinate-Q reductase	0.9	22	0.035	21.6	0.7	41.7

V_{max} is expressed as μmoles of dye reduced per mg SDH per minute at room temperature (23°). K_m is expressed in mM.

mentioned that the K_m^{PMS} for SDH is not affected by the presence of Q regardless of which artificial electron acceptor is used. In the case of succinate-Q reductase, K_m^{PMS} was found to be greatly affected by the presence of Q. The K_m value of the dye decreases proportionally to the amount of Q present in the system. Table I summerizes the data of K_m and V_{max} of SDH and succinate-Q reductase using different electron acceptors.

It is known that TTFA inhibits succinate oxidation when the dehydrogenase is in the "complex form" such as in cytochrome c reductase or submitochondrial particles, but not in soluble form which reacts only with artificial acceptors such as phenazine dyes (1). Reconstituted succinate-Q reductase is an ideal enzyme for experimentation to locate the TTFA locus. Figure 3A shows the inhibition of TTFA of reconstituted succinate-Q reductase activity. More than 90% of activity was inhibited by TTFA in micromolar concentration just like the enzyme bound to particles. On the other hand, the succinate to PMS reaction which measures the free dehydrogenase activity was completely unaffected by TTFA even if the concentration of inhibitor used was as high as 3 mM (See Fig. 1 of Ref. 1). Reconstituted succinate-Q reductase showed identical behavior to that of free SDH when PMS was used as the electron

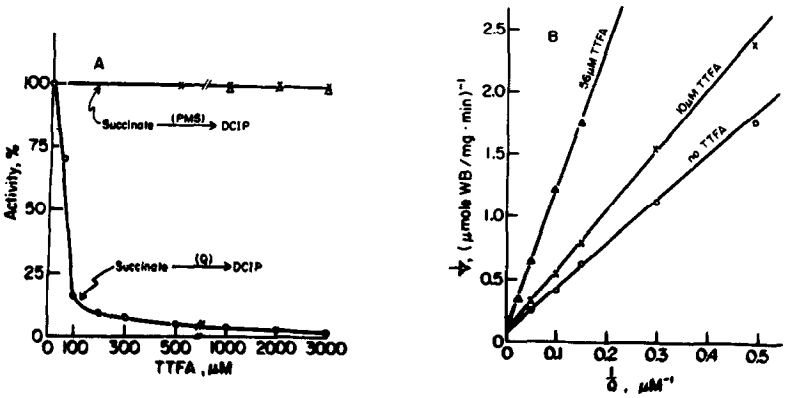

Fig. 3. Inhibition of thenoyltrifluoroacetone of reconstituted succinate-Q reductase activity. (A) was the titration of TTFA using Q (15 mM) and PMS (1.8 mM) as electron acceptors, and (B) was the double reciprocal plot of succinate-Q reactivity vs. Q concentration in the presence and absence of TTFA.

acceptor. When reconstituted succinate-Q reductase was assayed under various concentrations of Q in the presence of 10 μM and 56 μM TTFA, competitive inhibition was observed. Figure 3B shows the double reciprocal plots of the concentration of Q and the enzymic activity in the presence and absence of the inhibitor. An inhibitor constant, K_i, was calculated to be around 30 μM. It should be mentioned that all the concentrations of Q and TTFA used here refer to only apparent concentration. Because of the solubility of the reagents, the true concentrations might be smaller than these values.

Recently, two controversial articles (16, 8) have appeared regarding the enzymic activity, V_{max}, of SDH using WB and PMS systems for assay. Vinogradov et al. (8) reported that SDH catalyzes oxidation of succinate twice as fast using WB as electron acceptor as that using PMS; whereas, Ackrell et al. (16) found no significant difference in the rate of oxidation of succinate between these two dye systems. Our results automatically resolve the disparity.

On the other hand, our results give no support to the claim of the effect of "membrane environment" on SDH. Actually the so-called "membrane

environment" is just no more than the behavior exhibited after succinate dehydrogenase combines with QP-S. Since isolated QP-S contains only a small amount (< 20%) of phospholipid[4] and the protein was made in detergent, the possibility for the formation of succinate-Q reductase in a "membrane-like" structure of a "membrane environment" (17) after its recombination with SDH is not likely. The increase in the reduction rate of the dyes can be explained since QP-S is the native electron acceptor and thus oxidized succinate in a more effective way rather than arguing for the requirement of a "membrane environment" for such a reaction.

In conclusion, the success of reconstitution of succinate-Q reductase with well defined soluble SDH and QP-S not only resolves the "mystery" of "membrane environment" but also unequivocally defines the sites of TTFA inhibition and the reaction locus of DCIP (or related compounds) which themselves cannot directly interact with SDH.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institutes of Health and the American Heart Association, Northeastern New York Chapter, Inc.

REFERENCES

1. King, T. E. (1966) Adv. Enzymol. 28, 155-236.
2. Vinogradov, A. D., Gavrikova, E. V., and Goloveshkina, V. G. (1975) Biochem. Biophys. Res. Commun. 65, 1264-1269.
3. Kettman, J. R. (1967) Ph. D. Thesis, Oregon State University, Corvallis, Oregon.
4. Vinogradov, A. D., Ackrell, B. A. C., and Singer, T. P. (1975) Biochem. Biophys. Res. Commun. 67, 803-809.
5. King, T. E., Ohnishi, T., Winter, D. B., and Wu, J. T. (1976) in Iron and Copper Proteins (Yasunobu, K. T., Mower, H. F., and Hayaishi, O., eds.) pp. 182-227, Plenum Publishing Corp., New York.
6. King, T. E. (1963) J. Biol. Chem. 238, 4032-4036.
7. King, T. E. (1967) Methods Enzymol. 10, 322-331.
8. Vinogradov, A. D., Goloveshkina, V. G., and Gavrikova, E. V. (1977) FEBS Lett. 73, 235-238.
9. Yu, C. A., Yu, L., and King, T. E. (1977) Biochem. Biophys. Res. Commun. 78, 259-265.
10. Yu, C. A., Yu, L., and King, T. E. (1974) J. Biol. Chem. 249, 4905-4910.
11. King, T. E. (1967) Methods Enzymol. 10, 202-208.

[4] Accurate determination of lipid requires large amounts of the sample, thus 20% is the maximal value. More likely it is much smaller than the cited value when sufficient sample is available for assay.

12. King, T. E., Howard, R. L., Kettman, J., Jr., Hegdekar, B. M., Kuboyama, M., Nickel, K. S., and Possehl, E. A. (1966) in Flavin and Flavoproteins (Slater, E. C., ed.) pp. 441-481, Elsevier Publishing Co., Amsterdam.
13. Bernath, P., and Singer, T. P. (1962) Methods Enzymol. 5, 597-614.
14. Keilin, D., and King, T. E. (1960) Proc. Roy. Soc., London, Series B, 152, 163-187.
15. Mustafa, M. G., Cowger, M. L., Labbe, R. F., and King, T. E. (1968) J. Biol. Chem. 243, 1908-1918.
16. Ackrell, B. A. C., Coles, C. J., and Singer, T. P. (1977) FEBS Lett. 75, 249-253.
17. Ackrell, B. A. C., Kearney, E. B., and Singer, T. P. (1977) J. Biol. Chem. 252, 1582-1588.