Pyridoxal phosphate-induced dissociation of the succinate: ubiquinone reductase

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Treatment of the soluble ubiquinone-deficient succinate: ubiquinone reductase with pyridoxal phosphate results in the inhibition of the carboxin-sensitive ubiquinone-reductase activity of the enzyme. The inactivation is prevented by the soluble homolog of ubiquinone (Q_2) but is insensitive to the dicarboxylates interacting with the substrate binding site of succinate dehydrogenase. The reactivity of the pyridoxal phosphate-inhibited enzyme with different electron acceptors suggests that the observed inhibition is due to the dissociation of succinate dehydrogenase from the enzyme complex. The soluble succinate dehydrogenase was recovered in the supernatant after treatment of the insoluble succinate: ubiquinone reductase with pyridoxal phosphate. The data obtained strongly suggest the participation of amino groups in the interaction between succinate dehydrogenase and the ubiquinone reactivity conferring peptide within the complex.

Succinate: ubiquinone reductase *Respiratory chain* *Enzyme modification*

1. INTRODUCTION

The minimal structural unit capable of the succinate: ubiquinone reductase activity in mammalian mitochondria has recently been shown to contain, in addition to the two subunits of succinate dehydrogenase (EC 1.3.99.1) [1], one or two small polypeptides [2–6]. The water-soluble reconstitutively active succinate dehydrogenase interacts with a number of artificial electron acceptors, such as PMS [7], ferricyanide [8,9], and WB [10], but is unable to transfer electrons to ubiquinone, a natural electron acceptor for the membrane-bound enzyme. An active TTFA- or carboxin-sensitive succinate: ubiquinone reductase has been reconstituted by admixing the small peptide(s) with soluble succinate dehydrogenase [2–5], thus suggesting that the function of these peptides is to confer the reactivity of succinate dehydrogenase on ubiquinone.

Several lines of evidence including isolation and purification studies [11,12] indicate that the small peptide(s) are intrinsic parts of succinate: ubiquinone reductase and in the native membrane the enzyme apparently operates as a single oligomeric unit (complex II). Methods have been described for the dissociation of soluble succinate dehydrogenase from complex II or other particulate succinate: ubiquinone reductase preparations, including treatment with alkali [13], chaotropic resolution [14], and treatment with cyanide [15]. The nature of the forces which provide for the strong binding of succinate dehydrogenase and the ubiquinone reactivity conferring protein(s) remains obscure.

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Abbreviations: TTFA, thenoyltrifluoroacetone; carboxin, 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide; PMS, *N*-methylphenazonium sulfate; DCIP, 2,6-dichlorophenolindophenol; WB (Wurster's Blue), *N,N,N',N'*-tetramethyl-*p*-phenylenediimine radical; Q_2, ubiquinone having two isoprenoid units in position 6 of the quinone ring; PLP, pyridoxal phosphate

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Recently we have succeeded in developing a simple high-yield procedure for the preparation of soluble ubiquinone-deficient succinate:ubiquinone reductase from submitochondrial particles. Here, we present some data demonstrating that treatment of purified succinate:ubiquinone reductase with a lysine-specific reagent (pyridoxal phosphate) results in the dissociation of soluble succinate dehydrogenase from the complex. This finding suggests the importance of lysine residues for maintaining the enzyme complex structure and gives a clue for further chemically defined resolution of the succinate:ubiquinone reductase.

2. MATERIALS AND METHODS

Succinate:ubiquinone reductase was isolated from Keilin-Hartree heart muscle preparation [16], using a procedure developed in this laboratory (to be published). The preparation exhibits 4 major bands on SDS 12.5% polyacrylamide gels and contains (nmol or ngatom per mg protein): covalently bound flavin, 6; non-heme iron, 52; acid-labile sulfur, 50; and 2 mg bound Triton X-100 per mg protein. The enzyme catalyzes carboxin-sensitive Q_2 reductase and carboxin-insensitive PMS reductase at the rate of ~20 μmol succinate oxidized/min per mg protein at 25°C, pH 7.8. The soluble reconstitutively active succinate dehydrogenase [3] and oxaloacetate-free submitochondrial particles [17] were prepared according to the published procedures. The succinate:acceptor reductase activities were measured using PMS and DCIP [7], ferricyanide [8,9], WB [10] or Q_2 and WB [18] as acceptors according to the published procedures (for details see legends to the figures and table). The protein content was determined by the method of Lowry et al. [19] or Gornall et al. [20]. SDS-polyacrylamide gel electrophoresis was performed according to Laemmli [21], using 12.5% gels and stained with Coomassie brilliant blue (R-250).

3. RESULTS AND DISCUSSION

When soluble succinate:ubiquinone reductase was incubated with PLP, a time-dependent loss of the succinate:acceptor reductase activities was observed (fig.1). Only a slight decrease of the inhibition rate was found, when saturating concentrations of succinate or malonate were present, thus indicating that the target of PLP action is not the dicarboxylate binding site of the enzyme. In contrast, Q_2, the reactive homolog of ubiquinone, protects the enzyme against the PLP-induced inactivation. An almost complete loss of the carboxin-sensitive Q_2 reduction occurs after modification, whereas only partial inhibition of the succinate:PMS reductase activity was found. The data shown in fig.1 suggest that either the ubiquinone binding site of the enzyme is sensitive to PLP, or Q_2-protected deterioration of the enzyme complex occurs, when the amino groups are modified by PLP. It is worth mentioning that a

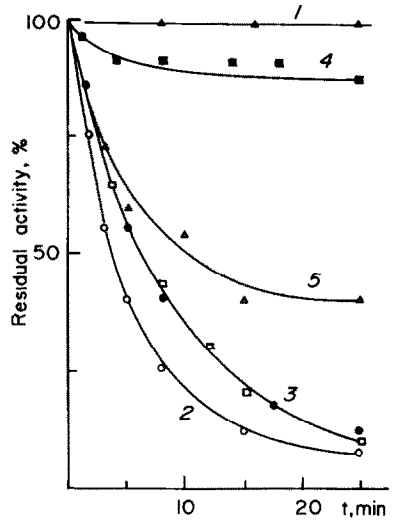

Fig.1. Inhibition of succinate:ubiquinone reductase by PLP. The enzyme (50 μg/ml) was incubated at 25°C in a mixture (pH 8.0) containing 20 mM phosphate, 0.1 mM EDTA (potassium salts), 20 mM PLP (except for control, curve 1); the time is indicated on the abscissa. Other additions (before PLP) were: curves 2 and 5, none; curve 3, 20 mM succinate or 1 mM malonate (potassium salts); curve 4, 20 μM Q_2 and 20 mM potassium succinate. A proper amount of the mixture was transferred to the assay mixture (pH 7.8) comprising 20 mM phosphate, 20 mM succinate, 0.1 mM EDTA (potassium salts) and 0.004% Triton X-100. The mixture was incubated for 10 min at 25°C and the assay was started by the addition of the acceptors (curves 1–4, 5 μM Q_2 and 30 μM WB; curve 5, 50 μM DCIP and 2 mM PMS). The rates of succinate oxidation were measured 30 s after addition of the acceptors. 100% corresponds to 20 μmol succinate oxidized/min per mg protein for PMS or Q_2 reduction.

Table 1

Comparison of the PLP sensitivities of different preparations of succinate dehydrogenase

Preparation	Specific activity[a] (μmol succinate oxidized/min per mg protein)		Inhibition (%)
	− PLP	+ PLP[b]	
Succinate:ubiquinone reductase[c]	17.0	1	94
Submitochondrial particles[c]	0.8	0.4	50
Soluble succinate dehydrogenase[d]	4.6 (2.9)	(2.9)	0

[a] The activities were measured in a mixture (pH 7.8) containing 20 mM phosphate, 20 mM succinate, 0.2 mM EDTA, 5 mM azide (potassium salts), 0.004% Triton X-100 and electron acceptors (70 μM WB for succinate dehydrogenase and submitochondrial particles, or 3 μM Q_2 and 20 μM WB for succinate:ubiquinone reductase)

[b] The samples were treated with 20 mM PLP for 30 min as described in fig.1

[c] The carboxin-sensitive activities are presented

[d] A considerable loss of the control activity occurs during aerobic incubation, therefore only the final levels (after 30 min, figures in parentheses) were compared for calculation of the inhibiting effect

similar inhibition pattern was observed when particulate succinate dehydrogenase was treated with cyanide [22], a reagent which is known to solubilize the enzyme and to destroy the iron-sulfur center of succinate dehydrogenase [15,23].

It seemed of interest to compare the inhibiting effect of PLP, using 3 different preparations of succinate dehydrogenase, namely, the ubiquinone-deficient soluble succinate:ubiquinone reductase, submitochondrial particles, in which the smaller peptides of complex II apparently form its bilayer intercalated part protected by endogenous ubiquinone [6], and the soluble reconstitutively active succinate dehydrogenase which contains no ubiquinone reactivity conferring components. As shown in table 1, the second preparation is much less sensitive to PLP than the ubiquinone-deficient enzyme, whereas succinate dehydrogenase itself is not sensitive to the inhibitor.

It has been shown that the reactivity of soluble succinate dehydrogenase towards ferricyanide can be used as a measure of functional intactness of the Hipip iron-sulfur center and that the reaction site for this acceptor is not accessible in the particulate enzyme preparations [3,4,8,9]. It might thus be expected that if the inhibitory effect of PLP is due to the dissociation of succinate dehydrogenase, the 'low-K_m ferricyanide' reaction site would appear in succinate:ubiquinone reductase after PLP treat-

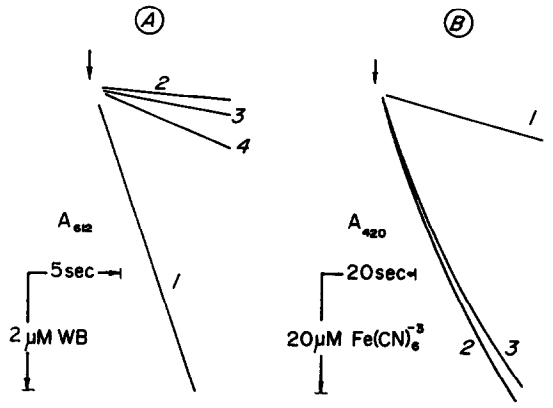

Fig.2. Reactivity of the PLP-treated enzyme towards different electron acceptors. The enzyme (0.7 mg/ml) was preincubated with or without 20 mM PLP for 20 min as described in fig.1. (A) The samples were added to the assay cuvette, incubated for 6 min at 25°C, and the reaction was started by the addition of 20 μM WB and 3 μM Q_2. Curve 1, no PLP was present in the preincubation mixture; curve 4, 20 mM PLP was present in the preincubation mixture; curves 3 and 2, as curves 1 and 4, respectively, no Q_2 was added to the assay cuvette. (B) The enzyme was preincubated as in (A) and the assay was started by the addition of 85 μM potassium ferricyanide. Curve 1, control; curve 2, PLP-treated enzyme; curve 3, as 2, 100 μM carboxin was present in the assay cuvette.

ment. Fig.2 demonstrates that intact carboxin-sensitive succinate: ubiquinone reductase does not interact with ferricyanide and that the carboxin-insensitive reactivity with this acceptor is induced by PLP treatment. The data presented in fig.2 provide strong, although indirect, indication that PLP inhibits succinate: ubiquinone reductase as a result of the succinate dehydrogenase dissociation. Direct evidence for such dissociation was obtained in experiments, where the succinate: ubiquinone reductase was made insoluble, treated with PLP and two subunits of succinate dehydrogenase were recovered in the supernatant (fig.3).

The inhibition of succinate: ubiquinone reductase reconstitution by some anions [4] as well as by treatment of soluble succinate dehydrogenase with fluorescamine [24] has been reported, thus suggesting the involvement of electrostatic interactions in the formation of the enzyme. Our data strongly reinforce the possibility that the ϵ-amino groups of lysine participate in the binding of hydrophilic succinate dehydrogenase to the mitochondrial membrane. The pH profile of succinate dehydrogenase solubilization [13] fits this proposal. In fact, solubilization of the enzyme in the presence of chaotropic anions, such as perchlorate [14], may also be the result of a strong electrostatic interaction between the positively charged lysyl residues of the enzyme and a large negatively charged chaotropic anion. Perhaps the most important observation reported here is that the reactive homolog of ubiquinone (Q_2) protects the enzyme against the PLP-induced dissociation. This protective effect offers a simple possibility for the investigation of the ubiquinone-binding site of the enzyme in terms of its location within the subunits and the interactions with other reactive centers of succinate: ubiquinone reductase.

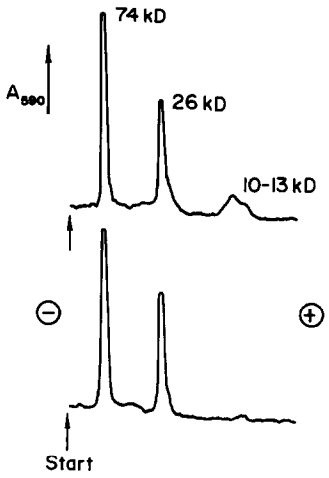

Fig.3. Structural dissociation of succinate: ubiquinone reductase induced by PLP. The enzyme (1 ml, 9.5 mg protein) was precipitated by the addition of 1 ml cold acetone, centrifuged for 5 min at 3000 × g, washed and suspended in 1 ml of the mixture (pH 8.0) containing 20 mM phosphate, 20 mM succinate, and 0.2 mM EDTA (potassium salts). One half of the suspension was incubated at 25°C with 20 mM PLP for 30 min, centrifuged and the supernatant was withdrawn for SDS-gel electrophoresis (lower curve). The other half was centrifuged, and the residue was subjected to electrophoresis (upper curve). About 100 μg protein was applied on each tube. No protein was detected in the final supernatant of the control (no treatment with PLP) sample. The molecular masses indicated were calculated, using a Pharmacia electrophoresis calibration kit containing proteins with molecular mass ranging from 14.4 to 94 kDa.

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REFERENCES

[1] Davis, K.A. and Hatefi, Y. (1971) Biochemistry 10, 2509–2516.
[2] Yu, C.A., Yu, L. and King, T.E. (1977) Biochem. Biophys. Res. Commun. 78, 259–265.
[3] Vinogradov, A.D., Gavrikov, V.G. and Gavrikova, E.V. (1980) Biochim. Biophys. Acta 592, 13–27.
[4] Yu, C.A. and Yu, L. (1980) Biochim. Biophys. Acta 593, 24–38.
[5] Hatefi, Y. and Galante, Y.M. (1980) J. Biol. Chem. 255, 5530–5537.
[6] Merli, A., Capaldi, R.A., Ackrell, B.A.C. and Kearney, E.B. (1979) Biochemistry 18, 1393–1400.
[7] King, T.E. (1963) J. Biol. Chem. 238, 4037–4051.
[8] Vinogradov, A.D., Gavrikova, E.V. and Goloveshkina, V.G. (1975) Biochem. Biophys. Res. Commun. 65, 1264–1269.
[9] Vinogradov, A.D., Ackrell, B.A.C. and Singer, T.P. (1975) Biochem. Biophys. Res. Commun. 67, 803–809.

[10] Vinogradov, A.D., Grivennikova, V.G. and Gavrikova, E.V. (1979) Biochim. Biophys. Acta 545, 141–154.
[11] Ziegler, D.M. and Doeg, K.A. (1962) Arch. Biochem. Biophys. 97, 41–50.
[12] Yu, L. and Yu, C.A. (1982) J. Biol. Chem. 257, 2016–2021.
[13] Wilson, D.F. and King, T.E. (1967) Biochim. Biophys. Acta 131, 265–279.
[14] Hanstein, W.G., Davis, K.A., Ghalambor, M.A. and Hatefi, Y. (1971) Biochemistry 10, 2517–2524.
[15] King, T.E., Winter, D. and Steele, W. (1972) in: Oxidation Reduction Enzymes (Åkeson, Å. and Ehrenberg, A. eds) pp.519–532, Pergamon, Oxford.
[16] Vinogradov, A.D. and King, T.E. (1979) Methods Enzymol. 55, 118–127.
[17] Kotlyar, A.B. and Vinogradov, A.D. (1984) Biochim. Biophys. Acta 784, 24–34.
[18] Grivennikova, V.G. and Vinogradov, A.D. (1982) Biochim. Biophys. Acta 682, 491–495.
[19] Lowry, O.H., Rosebrough, N.S., Farr, A.L. and Randall, R.S. (1951) J. Biol. Chem. 193, 265–272.
[20] Gornall, A.G., Bardawill, C.S. and David, M.M. (1949) J. Biol. Chem. 177, 751–766.
[21] Laemmli, U.K. (1970) Nature 227, 680–685.
[22] Giuditta, A. and Singer, T.P. (1959) J. Biol. Chem. 234, 661–671.
[23] King, T.E., Ohnishi, T., Winter, D.B. and Wu, S.T. (1976) Adv. Exp. Med. Biol. 74, 182–227.
[24] Yu, L. and Yu, C.A. (1981) Biochim. Biophys. Acta 637, 383–386.