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Involvement of a histidine residue in the interaction between membrane-anchoring protein (QPs) and succinate dehydrogenase in mitochondrial succinate-ubiquinone reductase

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The involvement of a histidine residue of the membrane-anchoring protein (QPs) fraction in reconstitution of succinate dehydrogenase to form succinate-ubiquinone reductase is studied by using a histidine-modifying reagent, diethylpyrocarbonate (DEPC). A maximum inactivation of 80% of reconstitutive activity is obtained when QPs is treated with 1 mM DEPC at 0°C for 30 min in 50 mM Tris-HCl (pH 7.0). DEPC also inactivates about 85% of intact succinate-ubiquinone reductase. The inactivation of succinate-ubiquinone reductase by DEPC is a result of the modification of essential histidine residues of succinate dehydrogenase. The inactivation is not a result of the modification of the histidine residue in QPs which is essential for interaction with succinate dehyrogenase because the QPs dissociated from the inactivated succinate-ubiquinone reductase is active in reconstitution with active succinate-dehydrogenase. Apparently, the essential histidine in QPs is shielded by succinate dehydrogenase and thus inaccessible to DEPC modification in succinate-ubiquinone reductase. The involvement of a histidine residue of QPs in interaction with succinate dehydrogenase is further evident by the presence of 553 nm shoulder on the α-absorption peak of reduced cytochrome *b*-560 (a characteristic of physical association of QPs with succinate dehydrogenase) in the DEPC-inactivated succinate-ubiquinone reductase. This shoulder disappears from a mixture of succinate dehydrogenase and DEPC-treated QPs when reduced with dithionite. About one histidine residue per molecule of QPs is modified in the DEPC-treated sample, suggesting that only one histidine residue is essential for interaction with succinate dehyrogenase. This essential histidine group is located in the smaller subunit (M_r 13000) of QPs.

Introduction

Mitochondrial succinate-ubiquinone (Q) reductase [1], which catalyzes the electron transfer from succinate to Q, can be resolved into two reconstitutively active fractions: a membrane-anchoring fraction, also known as QPs [2,3] a protein fraction [4], $CII_{3,4}$ [5], or a cytochrome *b*-560 fraction [6]; and a soluble succinate dehydrogenase [7–10]. Succinate dehydrogenase is composed of two protein subunits with molecular weights of 70000 and 27000. The larger subunit contains covalently bound FAD and the smaller subunit houses iron-sulfur clusters. QPs, which binds Q and converts succinate dehydrogenase into succinate-Q reductase, contains two protein subunits with molecular weights of 15000 and 13000. The M_r 15000 subunit is generally believed to be cytochrome *b*-560 and the M_r 13000 subunit is thought to be a Q reactivity-conferring protein [2]. However, when succinate-Q reductase was illuminated with the [^{3}H]arylazido Q derivative, both the 15000 and 13000 proteins were equally labeled [11]. It is possible that both proteins contribute to heme ligation in cytochrome *b*-560 as in the case of cytochrome *b*-559 [12] and both proteins participate in the formation of the Q-binding site.

Although EPR studies of *b* cytochromes in both succinate-Q and succinate-cytochrome *c* reductases [3] have confirmed the existence of cytochrome *b*-560 in succinate-Q reductase, the function of this cytochrome in this region of the electron transfer chain is not yet established. Isolated QPs preparations containing cytochrome *b*-560 have higher reconstitutive activities than

Abbreviations: Q. ubiquinone; QPs, a two-subunit protein fraction that converts succinate dehydrogenase into succinate-Q reductase; SDS, sodium dodecyl sulfate; HPLC, high-performance liquid chromatography; DEPC, diethylpyrocarbonate (also known as ethoxy formic anhydride); PMS, phenazine methosulfate.

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do QPs preparations containing no (or trace amounts of) cytochrome *b*-560. However, the direct participation of cytochrome *b*-560 in the catalytic function of succinate-Q reductase has been the subject of discussion because cytochrome *b*-560 in isolated succinate-Q reductase does not undergo redox change during catalysis and is present in a substoichiometric amount relative to succinate dehydrogenase in succinate-Q or succinate-cytochrome *c* reductase. The midpoint potentials of cytochrome *b*-560 in succinate-Q reductase and isolated QPs were reported to be −185 mV and −144 mV, respectively, which are in line with the fact that this cytochrome cannot be reduced by succinate [3]. However, the interaction of cytochrome *b*-560 with succinate dehydrogenase is demonstrated by the restoration of the absorption properties, redox potential and EPR characteristics of cytochrome *b*-560 during formation of a TTFA-sensitive succinate-Q reductase from QPs and succinate dehydrogenase [3].

The interaction between QPs and succinate dehydrogenase involves both hydrophobic and ionic forces. Hydrophobic interactions are suggested by the fact that succinate dehydrogenase can be detached from the membrane by chaotropic reagents [8]. The involvement of ionic interactions is supported by the observations that succinate dehydrogenase can be solubilized from the membrane by alkaline pH [7]; reconstitution of succinate-Q reductase from QPs and succinate dehydrogenase is ionic strength-dependent [13]; and an amino group in succinate dehydrogenase [14] and a carboxyl group in QPs [15] are involved in this reconstitution.

As a part of our continuing effort to elucidate the electron transfer mechanism in mitochondrial succinate-Q reductase we used diethylpyrocarbonate (DEPC), a histidine-modifying reagent, to examine the functional role of the modified histidine residue in QPs. We report herein the experimental conditions for DEPC modification of QPs, the effect of modification on reconstitutive activity and spectral properties QPs, the masking effect of succinate dehydrogenase against DEPC modification of QPs, and the location of the essential histidine residue in the subunits of QPs.

Experimental procedures

Materials. Triton X-100, diethylpyrocarbonate (DEPC), [^{14}C]DEPC, dichlorophenolindophenol (DCPIP), phenazine methosulfate (PMS), were obtained from Sigma. Bio-Beads SM-2 (20–50 mesh) were from Bio-Rad. Succinate-Q reductase [16], reconstitutively active and inactive succinate dehydrogenases [16], and two-subunit QPs [3] were prepared and assayed according to methods developed in this laboratory. Isolated two-subunit QPs was stirred with Bio-Beads SM-2 (1 g, wet weight, Bio-Beads/7 ml) for 2 h at 4 °C to remove most of the Triton X-100 present in the preparation before the addition of glycerol to a final concentration of 10%. The final QPs solution was frozen at −80 °C. Other chemicals were of the highest purity commercially available.

All enzymatic assays and absorption spectra analyses were performed either with a Cary spectrophotometer, model 219, or SLM-Aminco DW-2000, at 23 °C. Protein concentrations were determined by the method of Lowry et al. [17] in the presence of 1% SDS.

Reaction of DEPC with QPs. The frozen QPs solution in 50 mM Tris–HCl buffer (pH 7.0) containing 30 mM sucrose and 10% glycerol was thawed and the protein concentration was adjusted to 0.35 mg/ml with 50 mM Tris-HCl buffer (pH 7.0). To 0.1 ml aliquots of QPs were added 2 μl portions of acetonitrile containing various concentrations of DEPC. The final concentration of acetonitrile was kept below 2% to avoid denaturation of the enzyme. After incubation at 0 °C for 30 min the reaction mixture was treated with 50 mM L-histidine (pH 7.0) to quench the unreacted DEPC and was reconstituted with excess succinate dehydrogenase. Succinate-Q reductase activity was assayed after the reconstituted mixture was incubated at 0 °C for 20 min.

The number of histidine residues modified by DEPC is calculated from the absorbance difference at 240 nm between DEPC-treated and -untreated samples using a molar extinction coefficient of 3200 cm^{-1} [18] for *N*-carbethoxyhistidine.

Incorporation of [^{14}C]DEPC into QPs. [^{14}C]DEPC was diluted with unlabeled DEPC to a specific activity of 1.5 Ci/mol. 10-μl aliquots were withdrawn from the DEPC-treated sample at different time intervals and spotted on Whatman paper No. 3. When sampling was complete, the paper was developed with chloroform/methanol (2 : 1). The original spots in the paper were cut out, 5 ml of Insta-gel scintillation counting fluid was added, and radioactivity was determined in a Packard Tri-Carb scintillation analyzer, model 1900 CA. The counting efficiency was 50%.

The distribution of [^{14}C]DEPC between the two subunits of QPs was determined by measuring the radioactivity of each protein band after SDS-PAGE. The [^{14}C]DEPC-treated sample was dissociated with 1% SDS and 1% β-mercaptoethanol at 37 °C for 2 h before being subjected to SDS-PAGE [19]. The gels were sliced without staining and staining process and hydrolyzed with 2 M NH_4OH at 110 °C for 8 h before scintillation counting. The locations of protein bands were confirmed by staining an identical gel.

Results and Discussion

Effect of DEPC concentration on the reconstitutive activity of QPs

Fig. 1 shows the DEPC-concentration dependent inactivation of QPs. When QPs, in 50 mM Tris-HCl

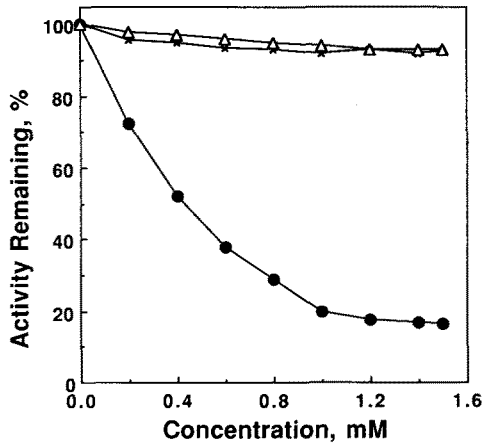

Fig. 1. Effect of DEPC concentration on the inactivation of QPs. To two sets of test-tubes: set A containing 50-μl aliquots of QPs (0.35 mg/ml, in 50 mM Tris-HCl (pH 7.0), and set B containing 50-μl aliquots of 50 mM Tris-HCl (pH 7.0) were added 1 μl portions of acetonitrile containing the indicated concentrations of DEPC. The mixtures were incubated at 0 °C for 30 min, added 1 μl aliquots of L-histidine were added (50 mM, pH 7.0), and incubation was continued for another 15 min. Aliquots of 50 μl of 50 mM Tris-HCl buffer (pH 7.0) were added to tubes of set A and aliquots of 50 μl of QPs solution (0.35 mg/ml, in 50 mM Tris-HCl (pH 7.0), were added to tubes of set B before the addition of 10 μl of succinate dehydrogenase (20 mg/ml, in 0.1 M phosphate buffer (pH 7.8) containing 20 mM succinate) to each tube. After 20 min incubation at 0 °C, succinate-Q reductase (●) and succinate-phenazine methosulfate (x) activities were assayed for set A, and succinate-Q reductase activity (△) was measured for set B. 100% activity represents the succinate-Q reductase activity (75 μmol succinate oxidized per min per mg of QPs) and succinate-phenazine methosulfate activity (65 μmol succinate oxidized per min per mg succinate dehydrogenase) in reconstituted succinate-Q reductase formed from untreated QPs and succinate dehydrogenase.

buffer (pH 7.0), is treated with various concentrations of DEPC at 0 °C for 30 min, the reconstitutive activity of QPs to form succinate-Q reductase decreases as the concentration of DEPC increases. A maximal inactivation of 80% is obtained with 1 mM DEPC. The presence of 2% acetonitrile in the reaction system, resulting from the addition of DEPC, causes no inactivation of QPs under the described conditions. Although the reason for the incomplete inactivation of QPs by DEPC even at the concentration higher than 1 mM is not known, the aggregation of QPs in its isolated form may be at least partial responsible. Reconstitution between QPs and succinate-dehydrogenase has been known not only to store the succinate-ubquinone reductase activity but also to deaggregate the QPs [13].

Since the activity of QPs is measured in reconstituted succinate-Q reductase and DEPC has been reported to inactivate succinate dehydrogenase by modifying essential histidine residues [20], it is necessary to establish that the inhibition is not due to inactivation of succinate dehydrogenase by the remaining DEPC using to inactivate QPs. It is highly unlikely that any DEPC remains in the systems after 30 min at 0 °C, since the half life of DEPC in Tris-HCl buffer was reported to be 1.25 min [21]. Moreover, a 50-molar excess of L-histidine was added at the end of reaction. Nevertheless, two control experiments are included. In one the succinate-phenazine methosulfate activity in reconstituted succinate-Q reductases formed from succinate dehydrogenase and DEPC-treated QPs was measured (Fig. 1, curve with triangles). In a second control experiment succinate-Q reductase activity was assayed after the addition of succinate dehydrogenase to solutions prepared by the addition of QPs to aliquots of 50 mM Tris-Cl buffer (pH 7.0) which had been incubated with DEPC at 0 °C for 30 min and treated with 50 mM L-histidine at the end of incubation (Fig. 1, curve with crosses). In both cases, a less than 7% decrease in activity is observed, confirming that no DEPC is left in the reaction system to inactivate succinate dehydrogenase during reconstitution. Thus, the activity loss (Fig. 1) observed is indeed due to inactivation of QPs by DEPC. The reconstitutive activity of the DEPC inactivated QPs is fully restored upon treatment with hydroxylamine.

Effect of incubation time and pH on the inactivation of QPs by DEPC

In the presence of 1 mM DEPC, maximal inactivation of 80% is reached after 20 min incubation at 0 °C (Fig. 2). A 30 min incubation time is used throughout this investigation to ensure completion of the reaction between DEPC and QPs and of the hydrolysis of residual DEPC.

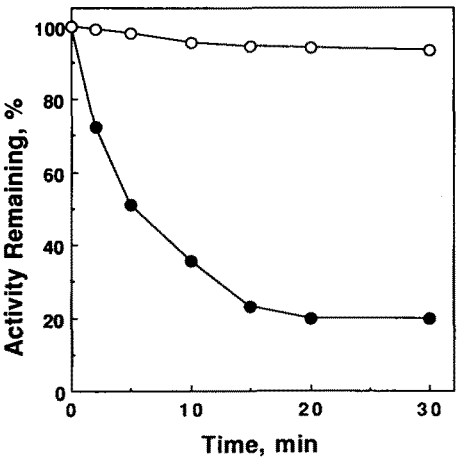

Fig. 2. Effect of incubation time on the inactivation of QPs by DEPC. To 1-ml aliquots of QPs (0.35 mg/ml, in 50 mM Tris-HCl buffer (pH 7.0), were added 10 μl of acetonitrile (○) and 10 μl DEPC (100 mM in acetonitrile) (●), respectively, and incubated at 0 °C. At the indicated time intervals, aliquots of 50 μl were withdrawn from each sample, added 5 μl of L-histidine (0.5 M in H_2O), and reconstituted with succinate dehydrogenase (35 μl, 4 mg/ml, in 0.1 M sodium/potassium phosphate buffer (pH 7.8), containing 20 mM succinate). Succinate-Q reductase activity was assayed 30 min after the reconstitution.

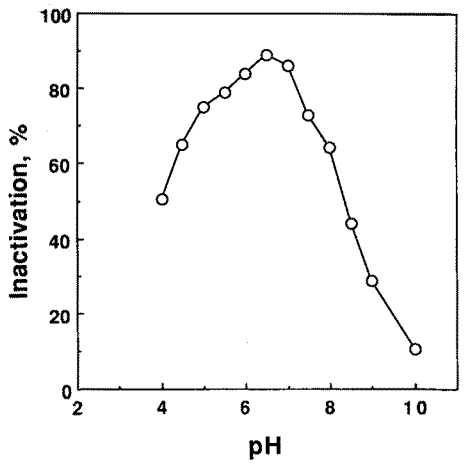

Fig. 3. pH-dependent inactivation of QPs by DEPC. 0.1 ml aliquots of QPs, 0.3 mg/ml, in 50 mM Tris-acetate at various pH levels were treated with and without 1 mM DEPC. The mixtures were incubated at 0°C for 30 min, added 0.4 ml of 100 mM phosphate buffer (pH 7.8), containing 20 mM succinate and 50 mM L-histidine, and reconstituted with succinate dehydrogenase (10 μl, 20 mg/ml, in 0.1 M phosphate buffer, (pH 7.8) containing 20 mM succinate). Succinate-Q reductase activity was assayed 20 min after the reconstitution. The percent activity remaining (○) was calculated based on untreated QPs samples at corresponding pH levels.

Fig. 3 shows that inactivation of QPs by DEPC is maximal at neutral pH. The pH profile of the inactivation of QPs by DEPC correlates well with the pH profile of the stability carbethoxyhistidine [22]. When the acidity or basicity of the reacting system increases, the extent of QPs inactivation by DEPC decreases. The slight pH variations during the reconstitution with succinate dehydrogenase before activity assays do not contribute to the pH-dependency of QPs inactivation by DEPC [13]. The subsequent experiments were performed at pH 7.0.

The inactivation of QPs by DEPC results from the modification of an essential imidazole group of histidine residues of QPs by DEPC. When the difference spectrum between intact and DEPC-inactivated QPs preparations is measured in the UV region (data not shown), an absorption peak is observed at 240 nm, indicating the modification of histidine and the formation of *N*-carbethoxyhistidine in the complex. Addition of hydroxyamine to the DEPC-treated sample abolishes the absorption peak at 240 nm region and restores the activity of QPs. Using a molar extinction coefficient of 3200 cm^{-1} at 240 nm for *N*-carbethoxyhistidine, about 1.2 mol of histidine per mol QPs are modified at maximal inactivation. Under the described conditions DEPC does not modify the tyrosine residues of QPs because no decrease in absorbance at 270 to 280 nm is observed.

Effect of succinate dehydrogenase on the inactivation of QPs by DEPC

Although it is clear that modification of a histidine residue in QPs by DEPC results in a loss of more than 80% of its reconstitutive activity, it is not clear whether this loss results from the modification of a histidine residue essential for catalytic activity of succinate-Q reductase or of a histidine residue essential for the interaction with succinate dehydrogenase. One way to distinguish between these possibilities is to investigate the effect of DEPC on isolated, functionally active succinate-Q reductase. Fig. 4 shows this DEPC concentration-dependent inactivation. When treated with increasing concentrations of DEPC, the succinate-Q reductase and succinate-phenazine methosulfate activities decrease. Maximal inactivations of 85% and 70% are observed for succinate-Q and succinate-phenazine methosulfate activities, respectively, when 3 mM DEPC is used.

Since the decrease in succinate-Q reductase activity correlates with that in phenazine methosulfate activity, it is highly probable that the observed loss of succinate-Q reductase results solely from inactivation of succinate dehydrogenase by DEPC [20,23]. To test this possibility, the DEPC-treated succinate-Q reductase preparation was adjusted to pH 10 under anaerobic conditions to dissociate succinate dehydrogenase from QPs. To this alkali-dissociated sample was quickly added either reconstitutively active succinate dehydrogenase or QPs, under anaerobic conditions before neutralization. As indicated in Table I, about 60% of succinate-Q reductase activity is restored in the succinate dehydrogenase-replenished sample. No activity restoration is found in the QPs-replenished sample. These results indicate that the histidine residue of QPs essential for reconstitution, is covered in intact succinate-Q re-

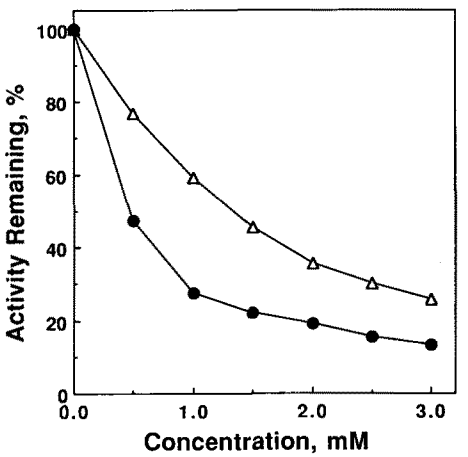

Fig. 4. Effect of DEPC concentration on the inactivation of succinate-Q reductase and succinate-phenazine methosulfate activities of succinate-Q reductase. To 50-μl aliquots of succinate-Q reductase (1.3 mg/ml, in 50 mM Tris-HCl buffer (pH 7.0)) were added 1 μl portions of acetonitrile containing the indicated concentrations of DEPC. After incubation at 0°C for 30 min, succinate-Q reductase (●) and succinate-phenazine methosulfate (△) activities were assayed. The 100% activity represents 16 and 17 μmol succinate oxidized per min per mg protein at 23°C for succinate-Q reductase and succinate-phenazine methosulfate activities, respectively.

TABLE I

Protective effect of succinate dehydrogenase on the inactivation of QPs by DEPC

To 1-ml aliquots of succinate-Q reductase (0.87 mg/ml, in 50 mM Tris-HCl (pH 7.0), were added 10 μl acetonitrile containing none (B) and 0.1 M DEPC (A). The mixtures were incubated at 0 °C for 30 min, 5 μl L-histidine (0.5 M) added, and assayed for succinate-Q reductase activity. After the activity was assayed, samples were flushed with argon and the following experiments were performed under anaerobic conditions. The pH of these two samples were adjusted to 10.0 with 1 M NaOH (40 μl). After incubation for 3 min at 0 °C, 200-μl aliquots were withdrawn from each sample, to which were added buffer only, reconstitutively active SDH, or QPs. The pH of the reconstituting solutions was titrated back to 7.0 with 2 M acetic acid (4 μl). Succinate-Q reductase activity was assayed after 30 min incubation at 0 °C. Treatment of reconstituted succinate-Q reductase formed from active succinate dehydrogenase and QPs (samples C and D) and a mixture of QPs and reconstitutively inactive succinate dehydrogenase (sample E) were the same as those described for succinate-Q reductase

Samples	Treatments	Succ.-Q specific activity
		μmol succ. oxid/ min per mg SQR
A SQR + DEPC	none	3.2
	OH^-, H^+	2.1
	OH^-, SDH, H^+	9.3
	OH^-, QPs, H^+	3.5
B. SQR	none	16.1
+ acetonitrile	OH^-, H^+	14.9
	OH^-, SDH, H^+	15.0
	OH^-, QPs, H^+	13.9
		μmol. succ. oxid/ min per mg QPs
C (SDH·QPs)	none	74.1
+ acetonitrile	OH^-, SDH, H^+	60.0
D (SDH·QPs)	none	20.7
+ DEPC	OH^-, SDH, H^+	33.3
E (Inactive SDH	none	4.2
+ QPs) + DEPC	OH^-, SDH, H^+	5.0

ductase, and thus is inaccessible to DEPC. The observed inactivation of succinate-Q reductase by DEPC results mainly from the modification of essential histidine residues of succinate dehydrogenase.

The protective effect of succinate dehydrogenase against inactivation of QPs by DEPC can only be observed when succinate dehydrogenase is physically associated with QPs. As indicated in the lower portion of Table I, inactivated succinate dehydrogenase cannot protect QPs from inactivation by DEPC because inactive succinate hydrogenase cannot form a physical complex with QPs [13]. A short term exposure of DEPC treated QPs to a high pH under the same condition has resulted in little QPs activity restoration, suggesting that no hydrolysis of carbethoxylhistidine occurs. This confirms that the observed protective effect of succinate dehydrogenase against inactivation of QPs by DEPC is due to the formation of a physical complex between the two moieties.

Incorporation and distribution of DEPC in the subunits of Qps

Fig. 5 shows the correlation between the loss of reconstitutive activity of QPs and its DEPC uptake. When QPs is incubated with 1 mM [^{14}C]DEPC at 0 °C for different lengths of time, the incorporation into the QPs protein is directly proportional to the loss of reconstitutive activity of QPs for the first 5 min of incubation. Beyond that, further activity decrease is very small, as is increased DEPC uptake. The break point for activity loss coincides with that for DEPC incorporation, suggesting that a specific histidine residue in QPs required for interaction with succinate dehydrogenase, is more reactive than other histidine residues. At maximal inactivation, about 1.2 mol of DEPC are incorporated into 1 mol of QPs protein. This result is comparable to that of estimated from the absorption increase at 240 nm.

Since isolated QPs contains two protein subunits, QPs-1 with $M_r = 15000$ and QPs-2 with $M_r = 13000$, it is of interest to know where this essential histidine residue is located. This can be determined by measuring the radioactivity distribution between the two subunits of QPs treated with [^{14}C]DEPC. As shown in Table II, most of the radioactivity was found in the smaller subunit, indicating that the histidine group essential for

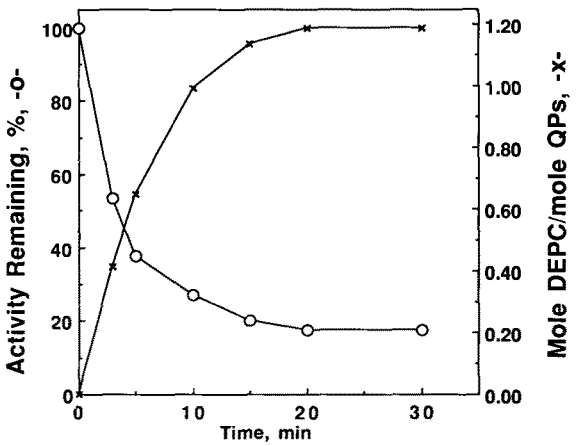

Fig. 5. Correlation between DEPC incorporation and inactivation of QPs. To 0.2-ml of QPs, 0.63 mg/ml, in 50 mM Tris-HCl buffer (pH 7.0) were added 2 μl of [^{14}C] DEPC (0.5 M in acetonitrile with specific radioactivity of 1.5 Ci/mol) and incubated at 0 °C. At indicated time intervals, 20-μl aliquots were withdrawn and reconstituted with succinate dehydrogenase. Succinate-Q reductase activity (○) was assayed after incubation at 0 °C for 20 min. For measurement of DEPC incorporation (×), 10 μl aliquot was withdrawn from the sample at indicated time intervals and spotted on Whatman No. 3 paper. The conditions for the paper chromatography and the method for radioactivity determination were detailed under Experimental procedures.

TABLE II

Distribution of [^{14}C]DEPC among subunits of succinate-Q reductase and QPs

Succinate-Q reductase (SQR) and QPs were treated with 1 mM [^{14}C]DEPC for 10 min at 0 °C and then quenched with 50 mM histidine. The mixtures were digested with 1% SDS and 1% β-mercaptoethanol and subjected to SDS-PAGE. 22 μg of SQR and 10 μg of QPs were applied to each gel. The molar concentration of SQR is calculated based on a purity of 6 nmol FAD pr mg protein; and QPs is based on molecular weight of subunits assuming a 90% purity of the preparation.

Subunit/ preparation	SQR		QPs	
	cpm	DEPC/protein	cpm	DEPC/protein
Fp	570	2.60	–	–
Ip	240	1.10	–	–
QPs-1	107	0.49	165	0.25
QPs-2	90	0.40	595	0.92

interaction with succinate dehydrogenase is located in QPs-2. The small amount of radioactivity found in QPs-1 is probably due to non-specific modification of histidine in the subunit. This would explain why more than 1 (1.2) mol histidine per mol protein is modified in order to reach the maximum inhibition.

Since this histidine is inaccessible to DEPC modification when QPs is complexed with succinate-dehydrogenase, it is important to determine the radioactivity distribution in succinate-Q reductase treated with [^{14}C]DEPC. In fact, the radioactivity is found primarily in the flavoprotein subunit, with a small amount in the iron-sulfur subunit of succinate dehydrogenase (Table II). The amount of radioactivity observed in QPs of [^{14}C]DEPC-treated succinate-Q reductase is rather small and is evenly distributed among its two subunits. These results support the conclusion that a histidine residue in QPs, which is inaccessible to DEPC in succinate-Q reductase, is essential for the interaction of QPs and succinate dehydrogenase.

Effect of DEPC on the spectral properties of QPs and succinate-Q reductase

Cytochrome *b*-560 in isolated QPs shows a symmetrical α-absorption with a maximum at 560 nm, while cytochrome *b*-560 in intact succinate-Q reductase shows α-absorption at 560 nm with a shoulder at 553 nm [3]. During reconstitution of QPs with succinate dehydrogenase to form succinate-Q reductase, the 553 nm shoulder reappears. Therefore, this 553 nm shoulder can be used to detect association of QPs with succinate dehydrogenase.

Fig. 6 shows the α-absorption spectra of cytochrome *b*-560 in DEPC-treated and untreated succinate-Q reductases, in reconstituted succinate-Q reductases formed from DEPC-treated and untreated QPs with succinate dehydrogenases. The DEPC-treated succinate-Q reductase, which lost over 70% of its activity, has the same absorption characteristics as those observed with untreated succinate-Q reductase, indicating that QPs remains associated with succinate dehydrogenase after DEPC treatment. This supports the observation that inactivation of succinate-Q reductase by DEPC results entirely from modification of essential histidine residues of succinate dehydrogenase. The histidine residue in QPs which is needed for interaction with succinate dehydrogenase is protected in succinate Q reductase. The α-absorption of cytochrome *b*-560 in DEPC-treated QPs is the same as that of untreated QPs; both show a symmetrical peak with a maximum at 560 nm, suggesting that modification of a histidine residue in the M_r 13000 subunit has no direct effect on the heme environ-

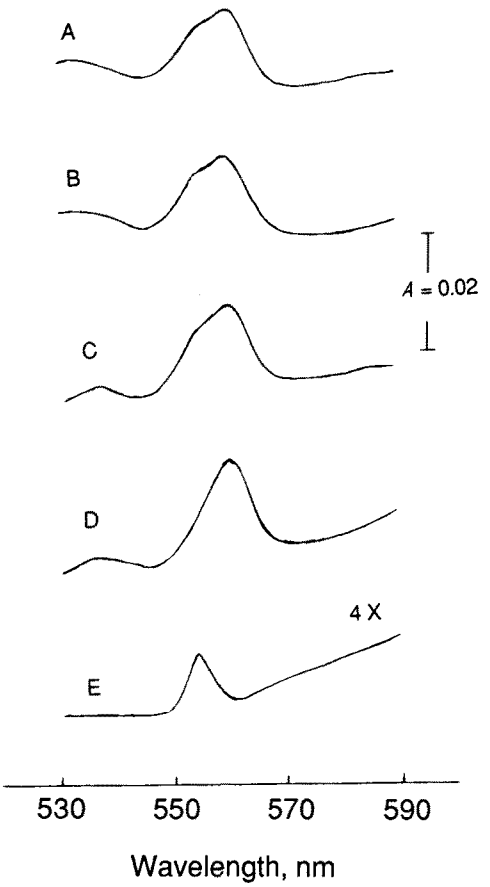

Fig. 6. Difference spectra of cytochrome *b*-560 in intact and DEPC-treated succinate-Q reductases, and in reconstituted succinate-Q reductases formed from DEPC-treated and -untreated QPs with succinate dehydrogenase in the α- and β-regions. (A) Dithionite-reduced succinate-Q reductase against oxidized sample. (B) Dithionite-reduced, DEPC-treated succinate-Q reductase against oxidized sample. (C) Dithionite-reduced reconstituted succinate-Q reductase formed from QPs and succinate dehydrogenase against oxidized sample. (D) Dithionite-reduced reconstituted succinate-Q reductase formed from DEPC-treated QPs and succinate dehydrogenase against oxidized sample. (E) Dithionite-reduced reconstituted succinate-Q reductase formed from QPs and succinate dehydrogenase against dithionite-reduced reconstituted succinate-Q reductase formed from DEPC-treated QPs and succinate dehydrogenase.

ment of cytochrome *b*-560. However, when succinate dehydrogenase is added to untreated QPs, the 553 nm shoulder appeared, while similar treatment of DEPC-treated QPs did not cause shoulder generation. These results strongly support the claim that a histidine residue in QPs is essential for interaction with succinate dehydrogenase. Information about the precise location of this histidine residue will not be available until the amino acid sequence of the M_r 13 000 protein is known.

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