Biochimica et Biophysica Acta 890 (1987) 47–54
Elsevier

BBA 42203

Subunit component and their roles in the sodium-transport NADH : quinone reductase of a marine bacterium, *Vibrio alginolyticus*

Maki Hayashi [a] and Tsutomu Unemoto [b]

Departments of [a] Enzymology and [b] Membrane Biochemistry, Research Institute for Chemobiodynamics, Chiba University, 1-8-1 Inohana, Chiba 280 (Japan)

(Received 8 July 1986)

Key words: Flavoprotein; NADH : quinone reductase; Respiratory chain; Subunit composition; Sodium ion transport; (Marine bacterium, *V. alginolyticus*)

The sodium-transport respiratory chain NADH : quinone reductase of a marine bacterium, *Vibrio alginolyticus*, was purified by high-performance liquid chromatography. The purified quinone reductase, which catalyses the reduction of ubiquinone to ubiquinol, was composed of three subunits, α, β and γ, with apparent molecular weights of 52 000, 46 000 and 32 000, respectively. The subunit β contained one molecule of FAD per molecule and catalysed the reduction of ubiquinone to ubisemiquinone. The subunit α contained FMN as a prosthetic group. The quinone reductase was reconstituted from α and βγ, but not from α and β, and the maximum activity was obtained at the equimolar amounts of FAD(β) and FMN(α). The molecular weight of quinone reductase complex was estimated to be 254 000, which corresponded to a dimer of αβγ complex or $\alpha_2\beta_2\gamma_2$. The subunit γ increased the affinity of β for ubiquinone-1. The reaction catalysed by FMN-containing α-subunit was essential for the generation of membrane potential in proteoliposomes and the coupling site of sodium pump in the quinone reductase was localised to this reaction step.

Introduction

A marine bacterium, *Vibrio alginolyticus,* has a respiratory chain-coupled Na^+ extrusion system functioning at alkaline pH [1,2]. The respiratory chain NADH : quinone reductase of this bacterium requires Na^+ for the maximum activity [3,4] and the Na^+-dependent quinone reductase has been shown to be tightly coupled to the extrusion of Na^+ [5,6]. The quinone reductase was partially purified from the membrane of *V. alginolyticus* and the reduction of ubiquinone to ubiquinol was found to proceed via ubisemiquinone radicals [7]. The reduction of ubiquinone to ubisemiquinone was catalysed by a flavoprotein, NADH dehydrogenase. Very recently, we have shown that in addition to FAD-containing NADH dehydrogenase, another flavoprotein containing FMN is required for the quinone reductase activity [8]. In this paper, we report the subunit composition and the role of subunits in the sodium-transport quinone reductase of *V. alginolyticus.*

Materials and Methods

Chemicals. Liponox DCH, an alkyl polyoxyethylene ether detergent having average alkyl chain and polyoxyethylene chain lengths of 10 and 8, respectively, was kindly supplied by Lion Co.,

Abbreviations: Q-1, ubiquinone-1; HQNO, 2-*n*-heptyl-4-hydroxyquinoline *N*-oxide; oxonol VI, bis(3-propyl-5-oxoisoxazole-4-yl)pentamethineoxonol.

Correspondence: T. Unemoto, Department of Membrane Biochemistry, Research Institute for Chemobiodynamics, Chiba University, 1-8-1 Inohana, Chiba 280, Japan.

Kanagawa, Japan. Ubiquinone-1 (Q-1) was kindly supplied by Eizai Co., Tokyo, Japan. 2-*n*-Heptyl-4-hydroxyquinoline *N*-oxide (HQNO) and L-α-phosphatidylcholine from soybean, Type IV-S, were obtained from Sigma. Bis(3-propyl-5-oxoisoxazole-4-yl)pentamethine-oxonol (oxonol VI) was obtained from Molecular Probes, Inc., OR.

Enzyme assays. NADH dehydrogenase was assayed at 30°C from the decrease in absorbance at 340 nm with menadione as an electron acceptor as described in Ref. 7. Quinone reductase was assayed at 30°C by following the formation of reduced Q-1 from the changes in absorption difference at the wavelength pair, 248–267 nm, as described [7]. Since the membrane and Liponox extract contain quinol oxidase activity, the quinone reductase of these fractions was assayed in the presence of 10 mM KCN.

One unit of activity is defined as the amount of enzyme catalysing the oxidation of 1 μmol NADH or the reduction of 1 μmol Q-1 per min.

High-performance liquid chromatography. Prepacked high-performance columns were obtained from Toyo Soda Manufacturing Co., Tokyo, Japan. Gel chromatography with a TSK-gel G3000SWG column (21.5 × 600 mm) and ion-exchange chromatography with a TSK-gel DEAE-5PW (21.5 × 150 mm) were performed as described previously [8].

Determination of molecular weight of quinone reductase complex. The molecular weight of the reductase complex was determined by means of a low-angle laser light scattering combined with high-performance gel chromatography as described by Maezawa and Takagi [9]. In this experiment, TSK-gel G4000SW (7.5 × 600 mm) and G3000SW (7.5 × 600 mm) columns were connected in series and it was equilibrated and developed with 20 mM Tris-HCl (pH 7.0)/0.1 M NaCl/5% glycerol/0.1 mM EDTA/0.1% Liponox. The eluate was monitored successively by an ultraviolet spectrophotometer UV-8000 (Toyo Soda), a precision differential refractometer RI-8000 (Toyo Soda) and by a low-angle laser light scattering photometer model LS-8000 (Toyo Soda). The molecular weight of protein moiety of the reductase complex was calculated according to Maezawa and Takagi [9]. The instrument constants were determined by use of monomer, dimer and trimer of bovine serum albumin as standards.

Preparation of proteoliposomes. Proteoliposomes containing quinone reductase were prepared by a cholate-deoxycholate dialysis procedure according to Sone et al. [10]. L-α-Phosphatidylcholine from soybean, Type IV-S, which contains about 40% of phosphatidylcholine, was used without further purification. The phospholipids were suspended in the medium containing 2% sodium cholate, 1% sodium deoxycholate, 10 mM Hepes-NaOH (pH 7.6) and 0.2 mM EDTA at the concentration of 50 mg/ml, and then it was subjected to sonic oscillation in a bath-type sonicator. The phospholipid solution (0.4 ml) and the enzyme solution (0.1 ml) were combined and the mixture was dialysed against 250 ml of solution containing 10 mM Hepes-KOH (pH 7.6)/2.5 mM $MgSO_4$/0.2 mM EDTA with stirring for 20 h at room temperature.

Measurement of membrane potential. The generation of membrane potential, positive inside, in proteoliposomes was monitored by an absorbance band shift of oxonol VI [11,12] at 625–587 nm with a Hitachi 557 two-wavelengths spectrophotometer.

Other methods. Flavins were identified by thin-layer chromatography on silica gel [8], and FAD and FMN contents were determined by the method of Faeder and Siegel [13]. SDS-polyacrylamide gel electrophoresis was performed by the method of Laemmli [14]. Gels were stained with Coomassie brilliant blue G-250 and then scanned by using a Shimazu dual-wavelegth chromato-scanner, CS-910, at 590–440 nm.

Kinetic constants and their standard errors were calculated as described previously [7].

Protein was determined by the method of Lowry et al. [15] with bovine albumin as a standard. The method of Bradford [16] was also employed for the determined of purified enzyme protein and the dye reagent was obtained from Bio-Rad Laboratories.

Results

Purification of quinone reductase

In our previous paper [7], we have shown that rechromatography of the quinone reductase on QAE-Sephadex leads to a partial dissociation of the reductase complex into subunits. Therefore, a

high-performance liquid gel chromatography was employed to remove impurities as much as possible and then the reductase complex was applied to an ion-exchange column.

The purification procedure has been outlined in our recent paper [8]. Briefly, the quinone reductase was extracted from the membrane fraction of *V. alginolyticus* with 1% Liponox in the presence of 10% glycerol as described in Ref. 7. The Liponox extract was applied to DEAE-Sephacel column and the reductase was eluted with 0.3 M NaCl (DEAE-Sephacel fraction). This fraction was applied to a TSK-gel G3000SWG gel column as described in Ref. 8. This step may be repeated to increase the specific activity of quinone reductase. The peak fraction was collected (SWG fraction) and then it was applied to TSK-gel DEAE-5PW.

Fig. 1 is the result of DEAE-5PW chromatography. The quinone reductase, which catalyses the reduction of ubiquinone to ubiquinol [7], was eluted at 61.5 min and the NADH dehydrogenase, which catalyses the reduction of Q-1 to ubisemiquinone [7], was eluted at 65.5 min. The relative amounts of the two enzymes varied depending on preparations and a prolonged storage of the sample increased the amount of NADH dehydrogenase.

Table I is a typical result of purification. The purified quinone reductase with the specific activity of 112 units/mg protein showed a high NADH dehydrogenase activity (181 units/mg protein). On the other hand, the NADH dehydrogenase with the specific activity of 255 units/mg protein

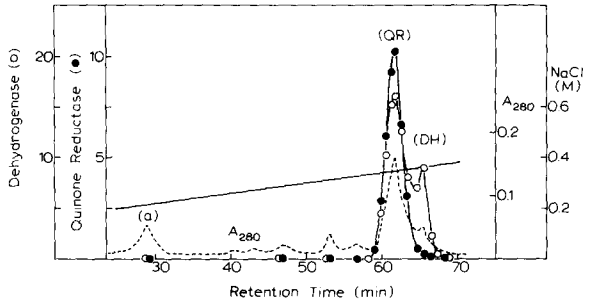

Fig. 1. TSK-gel DEAE-5PW chromatography. About 2.8 mg protein of second SWG fraction was applied to the column and it was eluted by a linear gradient from 0.1 to 0.55 M NaCl at the flow rate of 3 ml/min. The enzyme activities of eluate were measured by the NADH dehydrogenase assay (○) and by the quinone reductase assay (●) as described under Materials and Methods. The activity is expressed in units/ml. The dotted line denotes the absorbance of eluate at 280 nm. QR, quinone reductase; DH, NADH dehydrogenase.

showed only a slight quinone reductase activity. Further purification of NADH dehydrogenase with Bio-Gel HTP as described in Ref. 7 gave a highly purified enzyme with the specific activity of 390 units/mg protein, which showed no quinone reductase activity. Apparently, the NADH dehydrogenase was a component of the quinone reductase and was separated from the reductase complex during purification.

Subunit composition and flavin content of quinone reductase

Fig. 2 is the densitometric scans of stained bands of SDS-polyacrylamide gel electrophoresis. The three polypeptide components, designated as

TABLE I

PURIFICATION OF QUINONE REDUCTASE AND NADH DEHYDROGENASE FROM THE MEMBRANE OF *VIBRIO ALGINOLYTICUS*

Fraction	Protein (mg)	Quinone reductase assay		NADH dehydrogenase assay	
		(units)	(U/mg)	(units)	(U/mg)
1. Membrane	6440	8040	1.25	15485	2.40
2. Liponox extract	1054	3544	3.36	6038	5.73
3. DEAE-Sephacel	416	2958	7.11	5118	12.3
4. First SWG	35.5	1159	32.7	1983	55.9
5. Second SWG	14.0	753	53.8	1292	92.3
6. DEAE-5PW					
Quinone reductase	3.1	346	112	561	181
NADH dehydrogenase	1.2	14	11.7	306	255

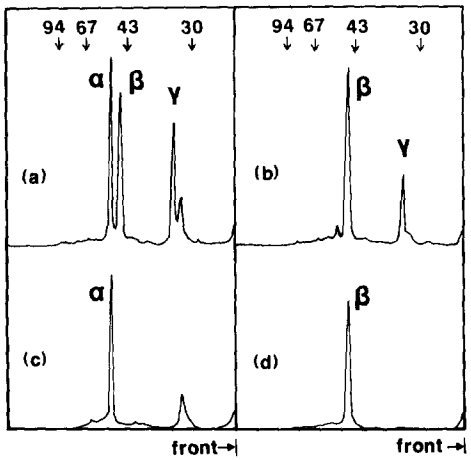

Fig. 2. Densitometer trace of SDS-polyacrylamide gel electrophoresis of purified enzyme on 10% acrylamide gel. The gel was stained and then scanned as described under Materials and Methods. The samples analysed, with specific activities in parentheses, were: (a) quinone reductase from DEAE-5PW (112); (b) NADH dehydrogenase from DEAE-5PW (255); (c) the α fraction from DEAE-5PW; (d) NADH dehydrogenase further purified by Bio-Gel HTP (390). The molecular weights of standard proteins are indicated in thousands.

α, β and γ, were concentrated in the quinone reductase (Fig. 2a). The plots of log relative mobilities of these subunits versus gel concentrations, namely Ferguson plots, showed no anomalous behavior and the apparent molecular weights of α, β and γ were estimated to be 52000, 46000 and 32000, respectively. The NADH dehydrogenase from DEAE-5PW (Fig. 2b) contained β and γ. However, the enzyme further purified by Bio-Gel HTP (Fig. 2d) contained a single polypeptide band β, indicating that the subunit β is NADH dehydrogenase.

The highly purified NADH dehydrogenase with the specific activity of 390 units/mg protein contained 21.3 nmol FAD/mg protein [8]. The molecular weight per FAD molecule was calculated to be 47000, which was very similar to that of β (46000). This means that β subunit contains one FAD molecule per molecule.

The subunit α was surveyed by means of the appearence of quinone reductase activity in combination with βγ as well as the detection of an α band on SDS-polyacrylamide gel electrophoresis. As shown in Fig. 1, the protein peak eluted at 29 min corresponded to α fraction. This fraction contained FMN as a prosthetic group at the concentration of 8.8–10.3 nmol/mg protein. As shown in Fig. 2c, this fraction still contained a broad polypeptide band as a contaminant, which appeared in front of γ.

Assuming that the color density of stained band is proportional to the amount of polypeptides, the weight ratio α : β : γ of the quinone reductase was calculated from the each peak area of Fig. 2a to be 1.25 : 1 : 0.84. If the quinone reductase was composed of equimolar quantities of α, β and γ, the weight ratio α : β : γ can be calculated to be 1.13 : 1 : 0.70 from their molecular weights. These values were very close to the estimated values. On the other hand, the NADH dehydrogenase from DEAE-5PW (Fig. 2b) contained β and γ in the ratio 1 : 0.46. The ratio of γ to β, however, varied from 0.24 to 0.54 depending on preparations, suggesting that γ was likely to be lost from the complex especially in the absence of α.

The purified quinone reductase contained FAD and FMN in equimolar quantities amounting to 6.6–7.2 nmol each flavin/mg protein. The flavin content corresponded to a minimum molecular weight of 150000–140000/each flavin molecule. Since the sum total of α + β + γ is 130000, these results also strongly suggested that the quinone reductase contains α, β and γ in equimolar quantities.

Determination of molecular weight of quinone reductase complex

The molecular weight of purified quinone reductase complex in 0.1% Liponox solution was determined by means of a low-angle laser light scattering combined with high-performance gel chromatography as described under Materials and Methods. As shown in Fig. 3, the quinone reductase complex was eluted as a single peak at a retention time of 35.5 min. The first peak observed only for the tracing of light scattering photometer (LS) was due to contaminated large particles or small air-bubbles. No other active species were detected in the eluate. The extinction coefficient at 280 nm for quinone reductase was estimated to be 1.85 $ml \cdot mg^{-1} \cdot cm^{-1}$. From the values of output of each detector, the molecular weight of protein moiety of the complex was calculated to be 254000. This value was twice that of α + β + γ, indicating

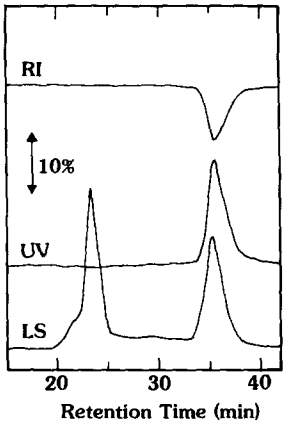

Fig. 3. Elution pattern of quinone reductase complex in 0.1% Liponox. The quinone reductase (83 μg) in 300 μl of the elution buffer was applied to the column and it was eluted at the flow rate of 0.8 ml/min. The eluate was monitored successively by the ultraviolet spectrophotometer at 280 nm (UV), the differential refractometer (RI) and by the laser light scattering photometer (LS). The tracings in the figure were adjusted so as to compensate time-lags among recordings of each monitor. Gain setting: ultraviolet monitor, 0.64 full scale; RI monitor, $128 \cdot 10^{-6}$; LS monitor, ×32.

that the active reductase complex exists as a dimer of $\alpha\beta\gamma$ or $\alpha_2\beta_2\gamma_2$ in 0.1% Liponox solution.

Reconstitution of quinone reductase

The α fraction by itself showed no enzyme activity, but the combination of α with βγ manifested the quinone reductase activity. Fig. 4 shows the reconstitution of quinone reductase from α and βγ. In this experiment, the amount of subunit was expressed in flavin content and the reductase activity was expressed in units/nmol FAD. With the increase in α (FMN), the activity increased and reached the maximum value at the equimolar amounts of FAD and FMN. The optimum ratio was not affected by the amount of βγ(FAD) used for the reconstitution. These results further indicated that the quinone reductase complex is composed of equimolar amounts of FAD and FMN. As shown in fig. 4, the combination of β with α gave no significant reductase activity and the presence of γ subunits was essential for the reconstitution of quinone reductase.

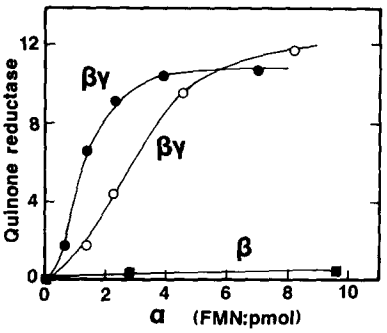

Fig. 4. Reconstitution of quinone reductase. βγ corresponding to 3.25 (●) or 6.5 (○) pmol FAD, or β corresponding to 3.6 pmol FAD (■), was preincubated with varied amounts of α for 30 min at 20°C in the medium containing 10 mM Tris-HCl (pH 7.9)/0.34 M NaCl/5% glycerol/0.1 mM EDTA/0.1% Liponox in a total volume of 20 μl. After that, 2 μl of the reaction mixture was used for the quinone reductase assay under the standard conditions. The activity is expressed in units/nmol FAD.

Effect of γ on the K_m value of β for Q-1

We have shown that Q-1 functions as the substrate of NADH dehydrogenase and quinone reductase and that the apparent K_m value for Q-1 of the former is considerably higher than that of the latter [7]. Therefore, the effect of γ on the K_m value of β for Q-1 was examined. In this experiment, preparations of βγ differing in the ratio γ : β were employed. As shown in Table II, the K_m value of β in the absence of γ was 11.7 μM, which decreased with the increase in the ratio γ : β. Although β and βγ used were prepared separately, the difference in the maximum velocity was small when expressed in units/nmol FAD. Apparently, γ subunit affected the affinity of β for Q-1. On the other hand, the K_m value of quinone reductase complex ($\alpha\beta\gamma$) for Q-1 was very low amounting to about 0.2 μM. Due to the

TABLE II

EFFECT OF γ ON THE K_m VALUE OF β FOR Q-1

The reaction was carried out under the conditions of quinone reductase assay except for the variation of the concentration of Q-1. The initial velocity was determined from the rate of NADH consumption. The ratio γ : β was determined from the ratio of color density of stained SDS-polyacrylamide gel.

Subunit	Ratio (γ/β)	K_m value for Q-1 (μM)	Maximum velocity (U/nmol FAD)
β	0	11.7 ±1.0	4.2 ±0.2
βγ	0.25	5.31±0.46	6.73±0.20
βγ	0.46	2.70±0.37	6.27±0.25
αβγ	0.87	0.19±0.17	21.6 ±1.1

absence of purified γ at present, the saturation level of γ could not be determined. However, the increase of γ to the level of quinone reductase seemed to decrease the K_m value of β close to that of αβγ. In contrast to β and βγ, since the αβγ complex catalyses the reduction of Q-1 to ubiquinol, the maximum velocity obtained with αβγ could not be directly comparable to those of β and βγ.

Generation of membrane potential in proteoliposomes

Proteoliposomes containing quinone reductase complex were prepared by cholate-deoxycholate dialysis procedure and the generation of membrane potential, positive inside, was monitored by the absorbance band shift of oxonol VI. As shown in Fig. 5, the addition of NADH in the presence of Q-1 induced the oxonol response suggesting the generation of Δψ, positive inside. The Δψ was collapsed by the addition of 1 μM valinomycin or 10 mM SCN^-. In a separate experiment, the quinone reductase activity was monitored under the same conditions. As shown in Fig. 5, the initial rate of quinol formation was 2.56 μM/min which corresponded to 28.4 units/mg protein. The oxonol response was dissipated with the consumption of Q-1, where all of Q-1 (7 μM) was converted to ubiquinol (Fig. 5). Further addition of Q-1 to the reaction mixture re-generated the Δψ. Although not shown here, when the reaction was carried out in the K^+-medium containing 50 mM K_2SO_4/10 mM Hepes-KOH (pH 7.6), no Δψ was generated. Under those conditions, the addition of 50 mM Na^+ induced the oxonol response as well as the ubiquinol formation. These Na^+-dependent reactions were completely inhibited in the presence of 2.5 μM HQNO. When menadione that accepts electrons from β(FAD) was used as an electron acceptor, no significant Δψ was generated (Fig. 5). Apparently the Δψ was generated in response to uniquinol formation and the Na^+-dependent and HQNO-sensitive reaction catalysed by α(FMN) directly participated in the generation of Δψ.

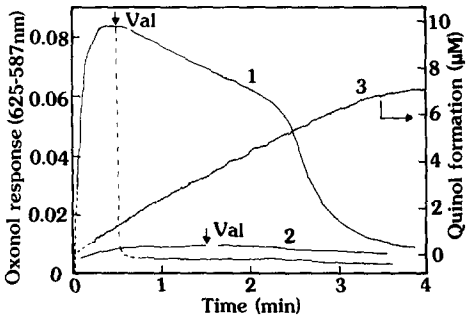

Fig. 5. Oxonol VI response of proteoliposomes containing quinone reductase complex. Proteoliposomes were prepared as described under Materials and Methods, which contained about 5 mM K^+ inside the vesicles. The stock solution of proteoliposomes contained 40 mg phospholipids and 36 μg quinone reductase (54 units/mg protein) per ml. The reaction mixture contained 10 mM Hepes-NaOH (pH 7.6)/2 mM $MgSO_4$/50 mM Na_2SO_4/1 μM oxonol VI/7 μM Q-1/0.1 mM NADH/5 μl proteoliposomes in a total volume of 2.0 ml. The reaction was started by the addition of NADH and the oxonol response was monitored at 625–587 nm (curve 1). In the curve 2, 25 μM menadione was used in place of Q-1. In a separate experiment, the quinol formation was monitored under the experimental conditions of curve 1 by following the absorbance changes at 248–267 nm (curve 3). Valinomycin (Val) was used at the concentration of 1 μM.

Discussion

Earlier studies in this laboratory [7] have demonstrated that the Na^+-dependent respiratory chain NADH : quinone reductase of *V. alginolyticus* catalyses the reduction of ubiquinone to ubiquinol via ubisemiquinone radicals. The formation of ubisemiquinone is catalysed by NADH dehydrogenase which has no specific requirement for Na^+ and is insensitive to HQNO. On the other hand, the latter reaction catalysing the formation of ubiquinol specifically requires Na^+ and is very sensitive to HQNO, that is a specific inhibitor of Na^+ pump [5]. In the present work, the highly purified quinone reductase was found to be composed of three polypeptide subunits α, β and γ with apparent molecular weights of 52000, 46000 and 32000, respectively (Fig. 2). Among these subunits, β corresponds to NADH dehydrogenase and contains one molecule of FAD per molecule as a prosthetic group. Since βγ complex shows no quinone reductase activity, the FMN-containing subunit α apparently participates in the reaction catalysing the quinol formation. This reaction is Na^+-dependent and HQNO-sensitive [7] and is essential for the generation of membrane potential in proteoliposomes (Fig. 5). Although a direct

measurement of Na^+ transport has not been made in this experiment, the coupling site of Na^+ pump in the quinone reductase may well be localized to the reaction catalysed by the α subunit from its Na^+-dependence and HQNO-sensitivity [5–7].

The attempt to isolate the γ-subunit in a pure state was unsuccessful at present and the molecular nature of γ remains to be established. However, it was apparent that γ is essential for the quinone reductase (Fig. 4). Since the presence of γ increases the affinity of β for Q-1 (Table II), γ seems to play an important role in the interaction of ubiquinone with the complex as well as the electron transfer from β(FAD) to α(FMN). King [17] has reported the participation of ubiquinone-binding proteins in the Complex I, II and III of mitochondrial respiratory chain. These proteins have been considered to stabilize ubisemiquinone and to dictate the location of Q sites in the redox reactions. Thus α is highly probable to be a kind of uniquinone-binding protein.

With respect to subunit composition, the quinone reductase contains α, β and γ in equimolar quantities. This conclusion was derived from the following findings: (1) the densitometric result of the stained SDS-polyacrylamide gel coincided with the presence of equimolar amounts of α, β and γ (Fig. 2); (2) the quinone reductase contained equimolar amounts of FAD and FMN, and the maximum activity was reconstituted at the equimolar amounts of FAD and FMN (Fig. 4); (3) the molecular weight of the complex per each flavin coincided with that of $\alpha + \beta + \gamma$ (130000). To estimate the molecular weight of active quinone reductase complex, a low-angle laser light scattering combined with high-performance gel chromatography was employed. This method was developed by Takagi and his coworkers and was successfully applied to membrane proteins [18]. The active quinone reductase is eluted as a single peak in the gel chromatography and the molecular weight of protein moiety is estimated to be 254000 (Fig. 3). Thus the active complex exists as a dimer of $\alpha\beta\gamma$ or as $\alpha_2\beta_2\gamma_2$ in 0.1% Liponox solution. Since no other active species are detected in the eluate, it is highly probable that this complex functions as a minimum active entity in the membrane also.

Ragan [19] proposed the electron-transfer pathways in Complex I of the mitochondrial respiratory chain, which cotnains FMN as a sole flavin and at least six iron-sulfur reaction centers. It has been postulated that the reduction-oxidation cycle of FMN via flavosemiquinone mediates the translocation of H^+ across the membrane in cooperation with iron-sulfur centers. On the other hand, Suzuki and King [20] reported that ubisemiquinone plays a central role in the translocation of H^+. Since the present enzyme system translocates Na^+ across the membrane [5,6], it is unlikely that either flavosemiquinone or ubisemiquinone directly functions as a carrier of Na^+. Thus an indirect-coupling redox pump mechanism as proposed for Complex III by Wikström and Krab [21] seems to be plausible to this enzyme system. Further studies are required to resolve these problems.

Acknowledgements

We are grateful to the technical assistance of Toyo Soda Manufacturing Co. in obtaining the result of Fig. 3. A part of this work was supported by a grant in aid from the Japanese Ministry of Education, Science and Culture.

References

1 Tokuda, H. and Unemoto, T. (1981) Biochem. Biophys. Res. Commun. 120, 265–271
2 Tokuda, H. and Unemoto, T. (1982) J. Biol. Chem. 257, 10007–10014
3 Unemoto, T., Hayashi, M. and Hayashi, M. (1977) J. Biochem. 82, 1389–1395
4 Unemoto, T. and Hayashi, M. (1979) J. Biochem. 85, 1461–1467
5 Tokuda, H. and Unemoto, T. (1984) J. Biol. Chem. 259, 7785–7790
6 Tokuda, H., Udagawa, T. and Unemoto, T. (1985) FEBS Lett. 183, 95–98
7 Hayashi, M. and Unemoto, T. (1984) Biochim. Biophys. Acta 767, 470–478
8 Hayashi, M. and Unemoto, T. (1986) FEBS Lett. 202, 327–330
9 Maezawa, S. and Takagi, T. (1983) J. Chromatogr. 280, 124–130
10 Sone, N., Yoshida, M., Hirata, H. and Kagawa, Y. (1977) J. Biochem. 81, 519–528
11 Bashford, C.L. and Thayer, W.S. (1977) J. Biol. Chem. 252, 8459–8463
12 Russell, J.T. (1984) J. Biol. Chem. 259, 9496–9507
13 Faeder, E.J. and Siegel, L.M. (1973) Anal. Biochem. 53, 332–336

14 Laemmli, U.K. (1970) Nature 227, 680–685
15 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
16 Bradford, M.M. (1976) Anal. Biochem. 72, 248–254
17 King, T.E. (1985) in Coenzyme Q (Lenaz, G., ed.), pp. 391–408, John Wiley & Sons, Chichester
18 Maezawa, S., Hayashi, Y., Nakae, T., Ishii, J., Kameyama, K. and Takagi, T. (1983) Biochim. Biophys. Acta 747, 291–297
19 Ragan, C.I. (1985) in Coenzyme Q (Lenaz, G., ed.), pp. 315–336, John Wiley & Sons, Chichester
20 Suzuki, H. and King, T.E. (1983) J. Biol. Chem. 258, 352–358
21 Wikström, M. and Krab, K. (1980) Curr. Top. Bioenerg. 10, 51–101