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Characteristic differences in the mode of quinone reduction and stability between energy-coupled and -uncoupled NADH-quinone reductases from bacterial respiratory chain

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Bacterial respiratory chain has two types of NADH-quinone reductase (NQR): one is energy-coupled (type-1) and the other has no energy-transducing capacity, that is, energy-uncoupled (type-2). Each of the NADH-reacting flavoprotein subunits of NQR-1 from *Escherichia coli* and the marine *Vibrio alginolyticus* reduced quinone to semiquinone radicals by the one-electron transfer pathway and was very sensitive to preincubation with NADH. On the other hand, the NQR-2 from these bacteria reduced quinone to quinol by the two-electron transfer pathway and was insensitive to preincubation with NADH. Since the NQR-1 from *E. coli* functions as a proton pump, whereas that from the marine *V. alginolyticus* functions as a sodium pump, the formation of semiquinone radicals as an intermediate is likely to be a common mechanism to functioning as either proton or sodium pump.

Respiratory chain; NADH-quinone reductase; One-electron transfer; Two-electron transfer; Proton pump; Sodium pump

1. INTRODUCTION

The membrane-bound respiratory chain of several bacteria such as *Escherichia coli* [1,2], a thermophilic *Thermus thermophilus* [3] and a marine *Vibrio alginolyticus* [4,5] contains two types of NADH-quinone reductase (NQR): one is energy-coupled (type-1) and the other has no energy-transducing capacity, that is, energy-uncoupled (type-2) (see [6] for a review). The energy-coupled NQR-1 from *E. coli* [1,2] and *T. thermophilus* HB-8 [3,7,8] function as a proton pump and are composed of multiple subunits containing noncovalently bound FMN and iron-sulfur clusters as prosthetic groups. In contrast, the energy-coupled NQR-1 from *V. alginolyticus* functions as a sodium pump [4,9] and is composed of three subunits, α, β, γ, containing both FAD and FMN as prosthetic groups [9–11]. On the other hand, the energy-uncoupled NQR-2 from *E. coli* is composed of single polypeptide containing noncovalently bound FAD as a prosthetic group [2,12]. We have demonstrated that the NADH-reacting FAD-containing β-subunit of NQR-1 from *V. alginolyticus* reduces quinones to semiquinone radicals by the one-electron transfer pathway [11]. After that, we purified the NADH-reacting FMN-containing subunit of NQR-1 (NFD) from *E. coli*, and it was found that the NFD is very sensitive, whereas the NQR-2 from *E. coli* is stable, to the NADH preincubation [2]. Furthermore, the NQR-2 purified from *V. alginolyticus* was found to reduce quinones by the two-electron transfer pathway [13]. At present, little is known about the differences in the reaction mechanism between NQR-1 and NQR-2 from these bacteria. Since the coupling ions of NQR-1 from *E. coli* and *V. alginolyticus* are different from each other, it is of interest to compare the mode of quinone reduction between the proton-translocating and sodium-translocating NQR-1s. This paper describes characteristic differences in the mode of quinone reduction and the sensitivity to the NADH preincubation between the energy-coupled NQR-1 and energy-uncoupled NQR-2 from *V. alginolyticus* and *E. coli*.

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2. MATERIALS AND METHODS

2.1. *Chemicals*

Ubiquinone-1 (Q1) was kindly supplied by Eizai, Tokyo. Other reagents used were of analytical grade.

2.2. *Enzyme preparations*

The FMN-containing NADH-ferricyanide dehydrogenase (NFD, the NADH-reacting flavoprotein subunit of energy-coupled NQR-1) and the FAD-containing NADH-quinone reductase (the energy-uncoupled NQR-2) from *E. coli* were prepared as described in [2]. The NFD with the specific activity for ferricyanide of 78 units/mg protein and the NQR-2 with the specific activity for Q-1 of 30 units/mg were used.

The FAD-containing β-subunit (45 units/mg for menadione) and the whole complex (α, β, γ) of sodium-translocating NQR-1 (58 units/

mg for Q-1) from *V. alginolyticus* were prepared as described in [9,11]. The NQR-2 (30 units/mg for Q-1) from *V. alginolyticus* was prepared as described in [13].

2.3. *Enzyme assays*

The NADH-quinone reductase activity was assayed at 30°C by following the formation of quinols as previously described [5,14]. The NQR-1, the β-subunit and the NQR-2 from *V. alginolyticus* were assayed under the conditions as described in [13]. The activity for menadione was assayed in the presence of 0.1 mM menadione in place of Q-1.

The NFD and the NQR-2 from *E. coli* were assayed under the conditions as described in [2] except that 0.1 mM NADH and 10 μM Q-1 were employed. The formation of ubiquinol-1 was measured from the changes in absorption difference at the wavelength pair, 242–270.5 nm by using the absorption coefficient of 9.6 $mM^{-1} \cdot cm^{-1}$ [2]. The consumption of NADH was measured from the decrease in absorbance at 340 nm by using the absorption coefficient of 6.81 $mM^{-1} \cdot cm^{-1}$ [2].

3. RESULTS AND DISCUSSION

3.1. *Sensitivity to the NADH preincubation*

We have demonstrated that the NADH-reacting flavoprotein subunit of NQR-1 (NFD) purified from *E. coli* is very sensitive, whereas the FAD-containing NQR-2 is resistant to preincubation with NADH [2]. Accordingly, the sensitivity to the NADH preincubation of the NQR-1 and the NQR-2 purified from *V. alginolyticus* were examined. When the NQR-1 was preincubated with NADH in the absence of electron acceptors, the activities for Q-1 and menadione progressively decreased with the preincubation time (Fig. 1A). The half-inactivation times ($T_{1/2}$) for Q-1 and menadione assays were 2 and 4 min, respectively. The activities of NQR-1, however, were unaffected by the preincubation in the absence of NADH. The purified β-subunit as measured with menadione as the electron acceptor was also very sensitive to the NADH preincubation with the $T_{1/2}$ of less than 2 min (Fig. 1B). The NQR-1 is composed of three subunits, α, β and γ, and the FAD-containing β-subunit accepts electrons from NADH and directly reduces menadione [5,11]. Thus, it was apparent that the sensitivity of NQR-1 to the NADH preincubation is mainly due to the inactivation of the NADH-reacting β-subunit in the NQR-complex.

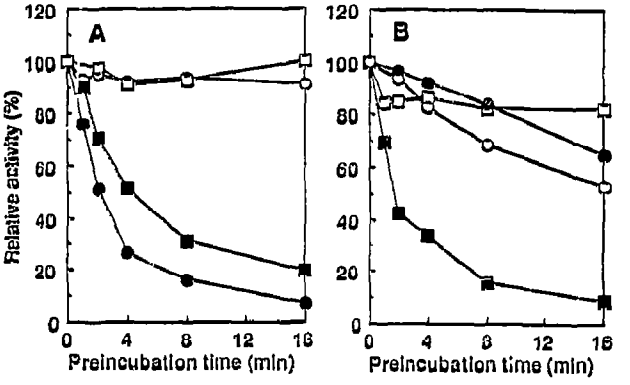

Fig. 1. Sensitivities to the NADH preincubation of the NQR-1, the β-subunit and the NQR-2 purified from *V. alginolyticus*. Each enzyme was preincubated at 30°C in the presence of 0.1 mM NADH for the indicated time in the absence of electron acceptors, and then the reaction was started by the addition of Q-1 or menadione. (A) After the preincubation of NQR-1 with NADH at the concentration of 0.2 μg/ml, the activity was assayed with Q-1 (●) or menadione (■). (B) The β-subunit (0.4 μg/ml) was assayed with menadione (■); the NQR-2 (0.2 μg/ml) was assayed with Q-1 (●). Open symbols represent the activities obtained by the preincubation with the electron acceptor as controls; the preincubation of the enzyme alone also gave the same results.

As shown in Fig. 1B, the NQR-2 purified from *V. alginolyticus* was relatively unstable to the preincubation even in the absence of NADH, and about 50% of the activity was lost in 16 min. However, since the rate of inactivation was rather retarded by the preincubation with NADH, the NQR-2 showed no specific sensitivity toward the NADH preincubation.

These results indicated that with the enzymes from *V. alginolyticus* the energy-coupled NQR-1 is sensitive, whereas the energy-uncoupled NQR-2 is insensitive, to the preincubation with NADH.

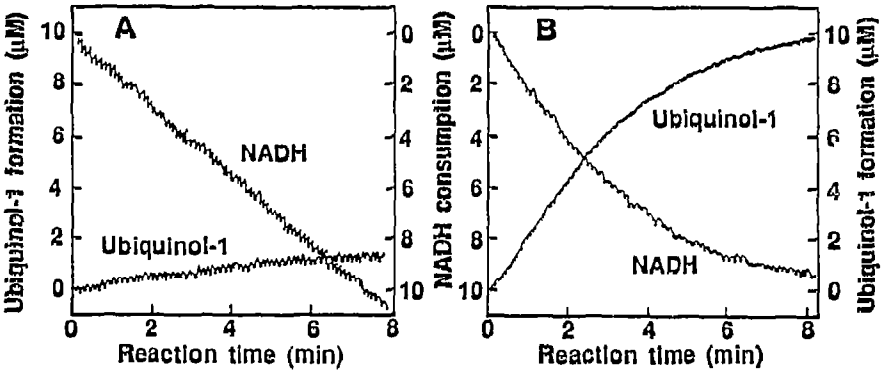

Fig. 2. The mode of quinone reduction by the NFD and the NQR-2 purified from *E. coli*. The reaction was carried out in the presence of 0.1 mM NADH and 10 μM Q-1, and the consumption of NADH and the formation of ubiquinol-1 were measured as described in 2.3. (A) NFD (6 μg/ml); (B) NQR-2 (0.1 μg/ml).

3.2. *The mode of quinone reduction by NFD and NQR-2 from* E. coli

We have shown that the NADH-reacting β-subunit of the sodium-translocating NADH-quinone reductase complex from *V. alginolyticus* reduces Q-1 and menadione to the corresponding semiquinone radicals by the one-electron transfer pathway [11]. Later, the energy-uncoupled NQR-2 purified from *V. alginolyticus* has been shown to reduce Q-1 and menadione to the corresponding quinols by the two-electron transfer pathway [13]. Thus, the mode of quinone reduction by the NFD and the NQR-2 purified from *E. coli* was examined. The NFD primarily reacts with ferricyanide, but menadione is also reduced with the activity of 4.6% of that for ferricyanide [2]. By using a large amount of NFD, Q-1 was reduced without the formation of ubiquinol-1, and NADH was consumed in excess of Q-1 (10 μM) present in the reaction mixture (Fig. 2A). Although not shown here, menadione was also reduced by the NFD without the formation of menadiol. On the other hand, the NQR-2 consumed NADH with the stoichiometric formation of ubiquinol-1 (Fig. 2B). These results clearly indicated that the NADH-reacting flavoprotein subunit of NQR-1 (NFD) catalyzes one-electron transfer and the NQR-2 two-electron transfer in *E. coli* also.

Although the energy-coupled NQR-1 from *V. alginolyticus* functions as the sodium pump and the energy-coupled NQR-1 from *E. coli* as the proton pump, the NADH-reacting flavoprotein subunit of NQR-1 from each bacterium exhibited very similar properties with respect to the sensitivity to the NADH preincubation and the mode of quinone reduction. Since each of the NADH-reacting flavoprotein subunit of NQR-1 reduces quinones by the one-electron transfer pathway, the formation of semiquinone radicals as an intermediate is very likely to be a common mechanism to functioning as either sodium or proton pump. Indeed, a semiquinone EPR signal has been detected in *T. thermophilus* HB-8 membranes containing proton-translocating NQR-1 [8]. In contrast, the energy-uncoupled NQR-2 from each bacterium was stable to the NADH preincubation and reduced quinones by the two-electron transfer pathway. Thus, the sensitivity of the flavoproteins to the NADH preincubation was apparently related to the mode of electron transfer. Further studies are required to make clear the details of the electron transfer pathways within the flavoproteins.

REFERENCES

[1] Matsushita, K., Ohnishi, T. and Kaback, H.R. (1987) Biochemistry 26, 7732–7737.
[2] Hayashi, M., Miyoshi, T., Takashina, S. and Unemoto, T. (1989) Biochim. Biophys. Acta 977, 62–69.
[3] Yagi, T., Hon-nami, K. and Ohnishi, T. (1988) Biochemistry 27, 2008–2013.
[4] Tokuda, H. and Unemoto, T. (1984) J. Biol. Chem. 259, 7785–7790.
[5] Unemoto, T. and Hayashi, M. (1989) J. Bioenerg. Biomembr. 21, 649–662.
[6] Yagi, T. (1991) J. Bioenerg. Biomembr. 23, 211–225.
[7] Meinhardt, S.W., Matsushita, K., Kaback, H.R. and Ohnishi, T. (1989) Biochemistry 28, 2153–2160.
[8] Meinhardt, S.W., Wang, D.C., Hon-nami, K., Yagi, T., Oshima, T. and Ohnishi, T. (1990) J. Biol. Chem. 265, 1360–1368.
[9] Hayashi, M. and Unemoto, T. (1987) Biochim. Biophys. Acta 890, 47–54.
[10] Hayashi, M. and Unemoto, T. (1986) FEBS Lett. 202, 327–330.
[11] Hayashi, M. and Unemoto, T. (1984) Biochim. Biophys. Acta 767, 470–478.
[12] Jaworowski, A., Campbell, H.D., Poulis, M.I. and Young, I.G. (1981) Biochemistry 20, 2041–2047.
[13] Hayashi, M., Miyoshi, T., Sato, M. and Unemoto, T. (1992) Biochim. Biophys. Acta 1099, 145–151.
[14] Unemoto, T. and Hayashi, M. (1979) J. Biochem. 85, 1461–1467.