Sequencing and the alignment of structural genes in the *nqr* operon encoding the Na^+-translocating NADH-quinone reductase from *Vibrio alginolyticus*

Maki Hayashi*, Kouji Hirai, Tsutomu Unemoto

Laboratory of Membrane Biochemistry, Faculty of Pharmaceutical Sciences, Chiba University, 1–33 Yayoi-cho, Inage-ku, Chiba 263, Japan

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Abstract **We previously cloned a part of *nqr* operon encoding the Na^+-translocating NADH-quinone reductase (NQR) from the marine *Vibrio alginolyticus* [Hayashi et al., FEBS Lett. 356 (1994) 330–332]. From its nucleotide sequences, four consecutive open reading frames (ORF) encoding the γ-subunit (27.7 kDa), two unidentified ORFs of 22.6 kDa and 21.5 kDa, and the β-subunit (45.3 kDa) were recognized. The gene encoding the α-subunit was located upstream, and together with the recent report by Beattie et al. [FEBS Lett. 356 (1994) 333–338], the *nqr* operon was found to be constructed from six consecutive structural genes, where *nqr1*, *nqr3* and *nqr6* correspond to the α-, γ-, and β-subunits, respectively, of the NQR complex.**

Key words: Na^+ pump; NADH-quinone reductase; Nucleotide sequence; *nqr* operon; *Vibrio alginolyticus*

1. Introduction

Gram-negative marine and moderately halophilic bacteria have a unique Na^+-translocating NADH-quinone reductase (NQR) complex as a first segment of the respiratory chain [1–5]. The enzyme complex purified from the marine *Vibrio alginolyticus* was composed of three subunits, α, β, and γ [6]. Recently we have cloned a part of *nqr* operon that contains the structural genes for the γ-subunit and β-subunit [7]. At the same time, Beattie et al. reported the presence of four consecutive open reading frames in the *nqr* operon containing the structural genes for the α-subunit and γ-subunit [8]. Our sequence data overlapped with those reported by Beattie et al. [8] at the structural gene for the γ-subunit. Thus we could construct the alignment of structural genes in the *nqr* operon. This paper reports the sequencing of the latter half of the *nqr* operon and the complete alignment of 6 ORFs in the *nqr* operon.

2. Materials and methods

2.1. Materials

The subclone, p3Cl, was prepared as described in [7]. The probe DNA was labeled with digoxigenin-dUTP with the DIG DNA labeling kit (Boehringer-Mannheim Biochemica), and was used for the Southern hybridization.

2.2. Analytical methods

Subcloning, restriction mapping, the preparation of template DNA, and other standard methods were performed as described in [9]. DNA was sequenced by the dideoxy method using Sequenase Version 2.0 DNA Sequencing Kit (Amersham). The primers used for sequencing were synthesized with Oligo1000 DNA Synthesizer (Beckman). A part of DNA was sequenced by an Automated Fluorescent DNA Sequencer (Shimazu DSQ-1000, Kyoto). Sequences were analyzed using the GENETYX-Mac.

3. Results

3.1. Sequencing of nqr genes

Fig. 1 shows the restriction maps and the strategies of nucleotide sequencing of p3Cl. The structural genes were renamed using numbers as discussed below. The nucleotide and deduced amino acid sequences of the cloned *nqr* genes are shown in Fig. 2, where 4,586 bps were sequenced. Four open reading frames (ORF) were detected. The first ORF (*nqr3*) starting from the nucleotide sequence of 965 had the predicted N-terminal and intermediate amino acid sequences determined from the γ-subunit [7]. Its sequence has been reported in [10] and registered under Accession Number D43958. It was composed of 256 amino acid residues with the M_r value of 27,703 Da, which was close to the experimental value of 32 kDa [6] determined by SDS-PAGE gel electrophoresis. The nucleotide sequence of the first ORF was in agreement with that of the *nqrC* gene reported by Beattie et al. [8] except for one point. In our nucleotide sequence, 1,513 and 1,514 were read as AT (Fig. 2), and thus the predicted amino acid residue was converted from R to W. The second ORF (*nqr4*) encoded 210 amino acid residues, which was in complete agreement with that of *nqrD*. Undoubtedly, our sequence data overlapped with that of Beattie et al. [8] at the first and the second ORFs. The third ORF (*nqr5*) encoded 198 amino acid residues. This protein was not identified in the purified NQR complex.

The fourth ORF (*nqr6*) encoded 407 amino acid residues with 45,274 Da, which was close to the value of 46 kDa for the β-subunit [6]. We previously prepared the probe B for the β-subunit, but it was inappropriate for the cloning of *nqr* operon [7]. By cloning a gene from the genome of *V. alginolyticus* with the probe B and sequencing its subclone, it was found that the amino acid sequence predicted an outer membrane maltoporin-like protein (unpublished results). Thus, the amino acid sequence for the β-subunit was incorrect. The region of the fourth ORF was confirmed to be indispensable for the expression of the β-subunit [7]. Moreover, the deduced amino acid sequence contained a common structure for NADH- or FAD-binding site. Thus, this ORF was assigned to be the structural gene for the β-subunit. Downstream of this ORF was a region of dyad symmetry followed by a T-rich region (Fig. 2), suggesting the end of *nqr* operon.

*Corresponding author. Fax: (81)(43) 255 1574.

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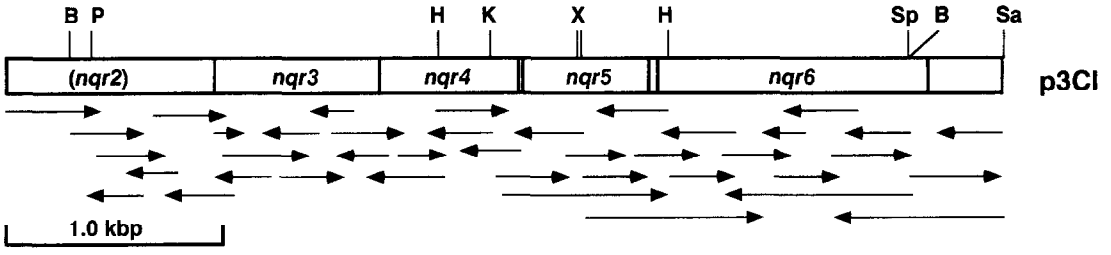

Fig. 1. Restriction maps and sequencing strategies of p3Cl. B, *Bgl*II; H, *Hin*dIII; K, *Kpn*I; P, *Pst*I; Sa, *Sal*I; Sp, *Sph*I; X, *Xba*I.

Table 1
Properties of four structural genes in the *nqr* operon

	nqr3	*nqr4*	*nqr5*	*nqr6*
Nucleotide residue	965–1,735	1,735–2,367	2,375–2,971	3,005–4,228
Amino acid residue	256	210	198	407
M_r (Da)	27,703	22,602	21,540	45,274
pI	4.38	9.17	7.00	4.55
Corresponding subunit	γ	unknown	unknown	β
Membrane-spanning helix	1	6–7	6	1–2

4. Discussion

We previously used *nqrA, B* and *C* to call the structural genes for the α-, β- and γ-subunits [7]. Beattie et al. reported the sequencing of four consecutive structural genes of *nqr* operon from *V. alginolyticus* and these genes were named as *nqrA, B, C* and *D* [8]. Among them, *nqrA* and *C* were identified as the structural genes for the α- and γ-subunits, respectively. The predicted N-terminal amino acid sequences coincided with our previous data [7]. Moreover, the nucleotide sequences of *nqrC* and *D* reported by Beattie et al. [8] coincided with those of the first and the second ORFs in the present paper. It is clear that the latter half of the *nqr* operon is sequenced in this paper. To avoid confusion, we recommend renaming the structural genes of the *nqr* operon with numbers starting from *ngrA*. Table 1 summarizes the properties of four structural genes in the *nqr* operon. In combination with the data of Beattie et al. [8], the *nqr* operon is apparently constructed from 6 ORFs, where *nqr1, nqr3* and *nqr6* encode the α-, γ- and β-subunits, respectively. The proteins encoded by *nqr4* and *nqr5* are very hydrophobic and have several predicted membrane-spanning helices. The amino acid sequences for proteins Nqr3–6 are not closely related to any subunits of H^+-translocating NADH-quinone reductase from prokaryotes [11,12] and eukaryotes [13,14], suggesting a unique structure of the Na^+-translocating NQR complex.

Our purified NQR complex was composed of three subunits, α, β and γ [6]. Using ubiquinone-1 as a substrate, the three subunits were essential for the Na^+-dependent NADH-quinone reductase activity. Therefore, the presence of three subunits is a minimum requirement for the catalytic activity. The role of subunits encoded by *nqr2, nqr4* and *nqr5* is unknown at present, and it is necessary to confirm their roles by reconstitution experiments.

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Fig. 2. The nucleotide and deduced amino acid sequences of the *nqr* genes. Each open reading frame was named as *nqr3,4,5* and *6* as shown in Table 1. Boxed amino acid sequences indicate the N-terminal and intermediate sequences determined for the γ-subunit. Underlined nucleotide sequence denotes putative Shine–Dalgarno sequences. Arrows represent a region of dyad symmetry adjacent to a poly(dT) region. The data are submitted under the Accession Number D49364.