Rapid report

The nirQ gene, which is required for denitrification of Pseudomonas aeruginosa, can activate the RubisCO from Pseudomonas hydrogenothermophila

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Abstract

Two putative ATP-binding proteins encoded in the gene cluster for the Calvin cycle of Pseudomonas hydrogenothermophila (cbbQ) and for the denitrification of Pseudomonas aeruginosa (nirQ) have been found to be similar. The cbbQ gene has been shown to activate the RubisCO from P. hydrogenothermophila in E. coli. The nirQ was functionally substituted for cbbQ. The nirQ gene restored the anaerobic growth and the NOR activity of the nirQOP mutant of P. aeruginosa, while the cbbQ gene did not. © 1998 Elsevier Science B.V. All rights reserved.

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Ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO; EC 4.1.1.39) is one of two unique enzymes required for the function of the Calvin–Benson cycle. This enzyme, which can be found in plants, algae, cyanobacteria, and most photo- and chemoautotrophic bacteria, is composed of eight large and eight small subunits (L8S8). The large subunit (Mr ~ 56000), encoded by the cbbL gene, contains the active site, while the precise role of the small subunit (Mr ~ 15000), encoded by the cbbS gene, is still unclear [1,2].

Pseudomonas hydrogenothermophila strain TH-1 is an aerobic, thermophilic, hydrogen-oxidizing bacterium and is a facultative chemolithotroph able to grow not only autotrophically using hydrogen, oxygen, and carbon dioxide, but also heterotrophically in organic media [3,4]. Our recent 16S ribosomal DNA gene sequence study indicates that this strain belongs to a new genus of β-subclass of Proteobacteria (N.R. Hayashi and T. Ishida, unpublished data). The strain is known to assimilate carbon dioxide via the Calvin–Benson cycle. The cbbQ and cbbO genes chase the RubisCO genes (cbbL, cbbS) [5,6]. CbbQ and CbbO are thought to play roles in the post-translational regulations of the RubisCO, because co-expression of cbbQ and cbbO with cbbLS in Escherichia coli affect the conformational state and the activity of RubisCO [6]. The cbbQ gene encodes a putative ATP-binding protein and is highly similar to the nirQ gene from Pseudomonas aeruginosa or Pseudomonas hydrogenothermophila.

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domonas stutzeri [5], as well as the norQ gene from Paracoccus denitrificans [7] or Rhodobacter sphaeroides [8]. The nirQ and norQ genes are located in the gene cluster dedicated to denitrification of these bacteria [7–10].

Bacterial denitrification is an anaerobic respiration in which nitrogen oxides are used as terminal electron acceptors, and the process has no direct relationship with the carbon dioxide assimilation via the Calvin–Benson cycle. Nitrate is usually dissimilated as gaseous dinitrogen or nitrous oxide in the denitrification pathway. The nirQ or norQ mutant strains could not grow anaerobically by denitrification [7,8,10]. The activity of nitric oxide reductase (NOR), one of the denitrification enzymes, was severely diminished in the nirQ/norQ mutant strains, in spite of the fact that the NOR protein was expressed in the cells [7,10]. This finding suggests that the nirQ or norQ gene product is thought to mediate the post-translational activation of NOR.

We constructed some expression plasmids using pUC and pACYC vectors. The details of the construction are shown in Fig. 1A. pYAH303 and pYAH304 are derivatives of pUC vectors designed to express cbbLS and cbbQ, respectively. pACYC184Cm−, used as a negative control of pYAH315 and pYAH321, was constructed by removing the EcoRI–ScaI fragment in the chloramphenicol resistance gene of pACYC184. The DNA amplified by PCR and treated by SphI and EcoRI was inserted into the ScaI and EcoRI sites of pACYC184 after being treated with T4 DNA polymerase (pYAH315). pACYC184 was digested by EcoRI and ScaI and was treated by the Klenow fragment. This 3.9-kb fragment was ligated by itself (pACYC184Cm−).

Fig. 1. Strains and plasmids used in this study. (A) Physical map of cbb genes of P. hydrogenothermophila. (B) Physical map of nirQ operon and nirQOP deletion mutant of P. aeruginosa. Strain PAO1 is a wild type and strain RM450 is a nirQOP deletion mutant of P. aeruginosa. P. aeruginosa RM450 was made by marker exchange mutagenesis. The methods for transformation and mutation of P. aeruginosa were described previously [15]. The pUC118 (Takara Shuzo, Tokyo, Japan), pUC119 (Takara Shuzo), pACYC184 [11], and pMMB67EH [16] were used for subcloning and also as expression vectors. Oligodeoxyribonucleotide primers were designed to prepare the cbbQ gene. The sequences of the primers were 5′-ATCCACGCATGCGGAGGCCTGCTATGG (primer 1, 5′ terminal of cbbQ gene including a putative ribosome-binding site and a SphI site) and 5′-TGGGTGAAATTCTCAGGCAAAGCGT (primer 2, 3′ terminal of cbbQ gene and an EcoRI site). The DNA fragment between primer 1 and primer 2 was amplified by PCR using pYAH304 DNA. The DNA amplified by PCR and treated by SphI and EcoRI was inserted into the ScaI and EcoRI sites of pACYC184 after being treated with T4 DNA polymerase (pYAH315). pACYC184 was digested by EcoRI and ScaI and was treated by the Klenow fragment. This 3.9-kb fragment was ligated by itself (pACYC184Cm−).
that the ability of NirQ from \( P. \) aeruginosa to activate the RubisCO from \( P. \) hydrogenothermophila was almost equal to that of CbbQ from \( P. \) hydrogenothermophila. SDS-PAGE of the cell extracts and immunological detection of RubisCO expressed in \( E. \) coli showed that the expression level of the enzyme protein was not affected by the existence of the \( cbbQ \) or \( nirQ \) gene (data not shown).

It is thought from the sequence result that NorQ from \( R. \) sphaeroides belongs to a family of proteins not exclusively required for denitrification [8]. CbbQ is thought to mediate the post-translational activation of RubisCO of \( P. \) hydrogenothermophila [6]. NirQ from \( P. \) aeruginosa activated the RubisCO from \( P. \) hydrogenothermophila to an extent equal to that achieved by CbbQ from \( P. \) hydrogenothermophila. Proteins of the CbbQ-type seem to form a new functional family to activate the protein post-translationally.

An operon including the \( nirQ \) gene is located between the structural gene for nitrite reductase (NIR) and NOR in \( P. \) aeruginosa (Fig. 1B) [9,12]. The \( nir-QOP \) mutant strain RM450 (Fig. 1B) used in this study was constructed by marker exchange mutagenesis with Tc resistance gene (\( tet \)) insertion. Insertion of the \( tet \) gene into the chromosome of the mutant was confirmed by Southern hybridization analysis (data not shown). Strain RM450 could not grow under anaerobic conditions with nitrate as an electron acceptor. NOR activity was severely diminished in the mutant strain (Table 2). The anaerobic growth and the NOR activity of RM450 were restored when the strain was transformed with the \( nirQ \) gene by plasmid pHA511. However, transformation with the \( cbbQ \) by plasmid pHA521 did not complement either the anaerobic growth or the NOR activity, indicating that \( cbbQ \) from \( P. \) hydrogenothermophila could not supplement the \( nirQ \) mutant in \( P. \) aeruginosa. SDS-PAGE of the cell extract of RM450 cultivated in aerobic condition detected the expression of CbbQ protein (data not shown).

We believe the \( nirQ \) gene is not necessary to fully

<p>| Table 1 |
| RubisCO activity in cell extract of ( E. ) coli( ^a ) harboring ( cbbLS, ) ( cbbLSQ ) or ( cbbLS-nirQ ) |</p>
<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Gene</th>
<th>RubisCO activity( ^b ) (U/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pYAH303+PACYC184Cm(^d )</td>
<td>( cbbLS )</td>
<td>0.42 ± 0.08</td>
</tr>
<tr>
<td>pYAH303+pYAH315</td>
<td>( cbbLS, cbbQ )</td>
<td>0.81 ± 0.15</td>
</tr>
<tr>
<td>pYAH303+pYAH321</td>
<td>( cbbLS, nirQ )</td>
<td>0.78 ± 0.02</td>
</tr>
<tr>
<td>pYAH315</td>
<td>( cbbQ )</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>pYAH321</td>
<td>( nirQ )</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

\( ^a \) \( E. \) coli was cultivated at 37\(^\circ\)C in LB medium.

\( ^b \) The average data were obtained from four repetitions of the experiments. The enzyme assay for RubisCO was performed by using \( \text{NaHCO}_3 \) at 50\(^\circ\)C as described previously [17]. One unit of enzyme activity is defined as the amount catalyzing the fixation of 1 \( \mu \)mol CO\(_2\) per min.

\( ^d \) The data are the average from three repetitions of the experiments. The activity was determined in the cell grown under the semi-aerobic conditions. Semi-aerobic cultivation was done in a 50-ml vial (70 ml total volume) containing 20 ml of LB medium. After inoculation, the vial was fitted with a butylrubber septum and an aluminum seal and then incubated at 30\(^\circ\)C. The enzyme assay for NOR was performed by using gas chromatography as described previously [18]. One unit of NOR activity is defined as the amount catalyzing the reduction of 1 \( \mu \)mol NO per min.

<p>| Table 2 |
| Properties of wild type and ( nirQ ) deletion mutant of ( P. ) aeruginosa |</p>
<table>
<thead>
<tr>
<th>Strain (genotype)</th>
<th>Plasmid</th>
<th>Anaerobic growth( ^{a} )</th>
<th>NOR activity( ^{b} ) (( \mu )U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA01 (wild)</td>
<td>+</td>
<td>1.39</td>
<td></td>
</tr>
<tr>
<td>RM450 (( nirQOP ))</td>
<td>−</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>RM450</td>
<td>−</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>RM450</td>
<td>pHA511 (( nirQ+ ))</td>
<td>+</td>
<td>2.22</td>
</tr>
<tr>
<td>RM450</td>
<td>pHA521 (( cbbQ+ ))</td>
<td>−</td>
<td>0.17</td>
</tr>
</tbody>
</table>

\( ^{a} \) The cells were grown anaerobically with nitrate as an electron acceptor.

\( ^{b} \) The data are the average from three repetitions of the experiments. The activity was determined in the cell grown under the semi-aerobic conditions. Semi-aerobic cultivation was done in a 50-ml vial (70 ml total volume) containing 20 ml of LB medium. After inoculation, the vial was fitted with a butylrubber septum and an aluminum seal and then incubated at 30\(^\circ\)C. The enzyme assay for NOR was performed by using gas chromatography as described previously [18]. One unit of NOR activity is defined as the amount catalyzing the reduction of 1 \( \mu \)mol NO per min.
activate RubisCO in *E. coli* cells. On the other hand, toxic NO concentration in the cell is maintained at a low level during denitrification [13], and consorted level of expression and function of NIR and NOR activities is necessary in order to avoid the accumulation of excess NO. The reactions of NIR and NOR are complex phenomenon and may be regulated by a complicated system, and the NirQ is believed to convert an inactive NOR to an active form [14]. It was thought that the *cbbQ* gene, instead of *nirQ* gene, could not activate the NOR completely, and this complexity is the reason why the *cbbQ* gene could not complement the *nirQOP* mutant of *P. aeruginosa*. In the *nirQ* mutant of *P. stutzeri* or the *norQ* mutant of *Pa. denitrificans*, the NOR activity was significantly reduced in spite of the fact that the NOR protein was produced [7,10], suggesting that the gene products of *nirQ* and *norQ* also have a role in the post-translational activation of the NOR protein.

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References