Purification and Characterization of Sulfide-Quinone Reductase, a Novel Enzyme Driving Anoxygenic Photosynthesis in Oscillatoria limnetica*

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An enzyme catalyzing sulfide quinone oxido-reduction (E.C.1.8.6.; SQR) has been purified in an active form, from thylakoids of the cyanobacterium Oscillatoria limnetica. It is composed of a single polypeptide of about 57 kDa. The catalytic activity of the purified enzyme is similar to the membrane-bound form in its kinetic parameters: apparent $K_m$ for sulfide equals 8 μM; $V_{max}$ of 100-150 μmol of plastoquinone-1 reduced/mg protein/h; quinone-substrate specificity; differential sensitivity to quinone analog inhibitors, the most potent of which being aurachin C ($I_50 = 7$ nm), and specific inducibility by sulfide. Taken together, they suggest that the purified SQR is the enzyme catalyzing anoxygenic photosynthesis in cyanobacteria.

The UV and visible absorption and fluorescence spectra of the purified SQR are typical of a flavoprotein. Both the absorption and fluorescence intensities are reduced by sulfide. The SQR activity is inhibited by KCN, a flavoprotein inhibitor. We have sequenced so far 29 amino acid residues of the SQR NH$_2$ terminus and found that from the second residue, this sequence contains the highly conserved fingerprint of the NAD/FAD-binding domain of many NAD/FAD-binding enzymes (Wierenga, R. K., Terpstra, P., and Hol, W. G. S. (1986) J. Mol. Biol. 187, 101-107). This suggests that the SQR enzyme is a flavoprotein which contains binding sites for sulfide and quinone and that the electron transfer between the two is mediated by FAD.

Of the many organisms performing organoxygen photosynthesis, only cyanobacteria can shift into anoxygenic bacteria-like photosynthesis, with sulfide as the electron donor in a photosystem I-dependent reaction (1-4). In Oscillatoria limnetica, the shift occurs after 2-3 h of incubation in the presence of sulfide and light and requires protein synthesis (5), which implies the involvement of sulfide-inducible protein(s) in anoxygenic photosynthesis. The induced cells perform sulfide-dependent CO$_2$ fixation (4-6), H$_2$O evolution (7), or N$_2$ fixation (8), depending on the growth and physiological conditions. The pathway of electrons, from sulfide to ferredoxin, is most likely common to all three reactions which are driven by photosystem I.

Sulfide-dependent photosynthetic electron transport has also been observed in cell-free systems prepared from induced O. limnetica cells (2, 9). In these preparations, sulfide reacts at two distinct sites with the electron transport system. One which is the physiologically important site, is inducible by, and has a high affinity to sulfide (apparent $K_m$ equals 20-40 μM). The reaction to NADP which involves this site is sensitive to inhibitors of the cytochrome $b_6$ complex (DNP-INT,1 stigma-tollin, NQNO, BAL, and 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (10, 11)), is coupled to a proton pumping (9), and is not lost by washing these membranes (9). Therefore, we have suggested that the inducible factor(s) which enables anoxygenic electron transport is a membrane protein, interacting at the PQ-$b_6$ site.

We have recently reported on a novel electron transport reaction from sulfide to externally added quinones, which is exclusively catalyzed by induced O. limnetica thylakoids in the dark. This sulfide-quinone oxidoreduction (SQR) reaction is most probably the initial step in sulfide-dependent anoxygenic photosynthesis. Both the light and dark reactions depend on a membranal sulfide inducible factor(s). The apparent $K_m$ for sulfide in both reactions is in the micromolar range, and the apparent $V_{max}$ for both reactions are also similar (12). Specific inhibitors of the cytochrome $b_6$ complex inhibit light-dependent electron transport from sulfide in induced whole cells as well as induced membrane preparations (2, 9). Some also inhibit the SQR activity in these membranes, suggesting that in both cases sulfide donates electrons to the PQ-$b_6$ part of the electron transport system (12).

We have solubilized and purified the SQR enzyme in an active form from O. limnetica thylakoids. The enzyme is a sulfide-induced flavoprotein composed of a 57-kDa monomeric polypeptide.

**Experimental Procedures**

Cyanobacterial Growth, Induction, and Membrane Preparation

O. limnetica was grown aerobically in 1-liter batch cultures. The induction of anoxygenic photosynthesis was carried out for 6 h and thylakoid membranes prepared (12).

1 The abbreviations used are: DNP-INT, 2,4,5-trinitro-3-methyl-2'morpholinoethanesulfonic acid; HPLC, high performance liquid chromatography; CHAPS, 3-[N-(cholamidopropyl)-dimethylammonio]-propane-sulfonate.

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Sulfide-Quinone Reductase from O. limnetica

Purification of the SQR

Purification was performed at 4 °C, except for the HPLC steps, which were conducted at room temperature. The purification protocol was as follows:

Membrane Wash—Frozen (-80°C) membranes (25–100 mg of chlorophyll) were thawed and resuspended in a buffer containing 5 mm EDTA-Na, 5 mm MES-Na, pH 6.7, at 0.1 mg of chlorophyll/ml. The membranes were centrifuged for 10 min at 18,000 x g, resuspended in the same buffer, homogenized (by a glass-Teflon tissue homogenizer), diluted to 0.1 mg of chlorophyll/ml, and centrifuged for 25 min at 38,000 x g. This washing step was repeated twice. The membranes were then washed with a buffer containing 20 mm HEPEES-Na, pH 7.2, 0.4 mM sucrose, and 3 mM MgCl₂, homogenized as above, diluted to 0.2–0.3 mg of chlorophyll/ml, and centrifuged for 10 min at 16,000 x g.

Solubilization—The washed membranes were resuspended in a buffer containing 20 mm HEPEES-Na, pH 7.2, 0.4 mM sucrose, 10 mM MgCl₂, to a concentration of about 0.75 mg of chlorophyll/ml and homogenized. Sodium cholate and dodecyl maltoside were added sequentially to a final concentration of 10 and 25 mM, respectively. After 30 min of incubation on ice, the membranes were centrifuged for 30 min at 39,000 x g, and the orange supernatant collected (DM supernatant).

Ammonium Sulfate Fractionation—The DM supernatant was fractionated by ammonium sulfate between 55 and 75% saturation. The 75% pellet (P75) was then washed twice with 10 ml of 75% ammonium sulfate, 5 mm MES-Na, pH 6.0, and 5 mm DM. The last pellet was resuspended and homogenized in 50 mm MES-Na pH 6.0, 100 mm NaCl, 10 mM MgCl₂, and 5 mm DM, frozen, and stored in liquid nitrogen and stored at -70 °C.

High Performance Liquid Chromatography—HPLC was performed on a Merck Hitachi system (Japan) composed of a L-6200 pump and a Hitachi gel filtration column (TSK-gel, type G3000 SW column, 2.15 x 25 cm) at a flow rate of 0.5 ml/min. Fractions (0.3 ml) were collected, assayed, and stored at -70 °C. The four most active fractions from the first gel filtration were combined after washing the membranes (12). Reaction mixture routinely contained 10 mM MgCl₂, 0.1 mg of chlorophyll/ml, and was fully active after 24 h of incubation at 4 °C.

SDS-gel electrophoresis was carried out either according to Laemmlli (13) or Schagger and von Jagow (14) and silver-stained (15). For membranes, SDS concentration in the sample buffer was 4%.

[^57S]Methionine Labeling

O. limnetica cells were incubated for 2 h in the presence of[^57S]Methionine (0.6 μM; 75 μCi/ml) under inducing as well as non-inducing conditions. 

Preparation of Antibodies

Active SQR fractions from the hydrophobic column (about 200 μg of protein) were further purified on 10–15% gradient polyacrylamide SDS gel (Laemmlli) and Coomassie-stained. The 57 kDa major band, separated from the minor contaminant, was cut out and homogenized in 10 mM potassium phosphate, pH 7.4, 150 mM NaCl. The homogenate was mixed with an equal volume of complete Freund's adjuvant and injected intradermally into two rabbits. A boost of a similar sample was injected after 18 days, and the rabbits were bled weekly for the next 3 weeks.

Sequencing the Amino Terminus of the Purified SQR

A sample of the most active fraction obtained after the last purification step (gel filtration) was further purified by SDS-polyacrylamide gel electrophoresis and electroelution. It was then adsorbed on to a polyvinylidene difluoride membrane and sequenced in an Applied Biosystems 475/A Protein Sequencer.

Other Assays

Sulfide concentration was determined by the methylene-blue method (17). Chlorophyll a was extracted from thylakoids with 100% methanol and measured (18). Protein was determined by a modified Lowry method (19).

Materials

DNA-INT, aurachin-C, and NNQNO were kindly donated by Dr. A. Trebst, Ruhr University, Dr. G. H. Höfe, Braunschweig, and B. L. Trumpower, Dartmouth Medical School, respectively. PQ-1 was synthesized according to Rich et al. (20). DM was purchased from Sigma. Cholate was three times crystallized (21).[^57S]Methionine was purchased from Amersham (United Kingdom).

RESULTS AND DISCUSSION

Solubilization of SQR—We have previously shown that thylakoids from sulfide-induced O. limnetica cells exhibit SQR activity, which is not released by washing the membranes (12). Therefore the induced thylakoids were washed three times with 5 mM EDTA-Na prior to SQR isolation. This procedure removed most of the peripheral SQR proteins, including about 99% of the phycobiliproteins (data not shown).

Various treatments were tested for their ability to release the SQR from the washed membranes. Mild sonication, or treatment with Na-cholate (up to 0.6%), failed to solubilize SQR activity. Incubation of the membranes with 30 mM of either CHAPS, MEGA-9 (nonanoyl-N-methylglucamide), or 1% Triton X-100 released the SQR from the membranes, but the activity decayed within a few hours. The total SQR activity released by DM (25 mM) was about 50% higher than with octyl glucoside (30 mM) and was fully active after 24 h of incubation at 4 °C. The presence of cholate improved the specificity of the solubilization by DM as judged by the markedly lower amount of chlorophyll, which was coextracted. On the other hand, the presence of ammonium sulfate (10%), previously reported to...
Sulfide-Quinone Reductase from O. limnetica

The purification steps of SQR are described in the text.

<table>
<thead>
<tr>
<th>Purification Fraction</th>
<th>Protein (mg)</th>
<th>Chlorophyll</th>
<th>Activity (μmol PQ-1 red/h)</th>
<th>Specific Activity (μmol PQ-1 red/mg protein h)</th>
<th>Purification Factor</th>
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<td>757</td>
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<td>Detergent extract (DM-supernatant)</td>
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<td>1.8</td>
<td>1,610</td>
<td>7.4</td>
<td>7</td>
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<tr>
<td>Ammonium sulfate (P-75)</td>
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<td>0.8</td>
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<td>2nd gel filtration</td>
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<td>80</td>
<td>113.3</td>
<td>105</td>
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</table>

![Fig. 1. Elution profiles of the HPLC purification steps.](image)

The optimized conditions for the solubilization of the SQR thus included 25 mM DM, 10 mM cholate, and O. limnetica thylakoids containing 0.75 mg of chlorophyll/ml. Under these conditions only 30% of the total protein, 5% of the chlorophyll, but most of the carotenoids were released (Table I). The total extracted SQR activity was higher than in the membranes. It is thus suggested that DM specifically releases the enzyme, as shown in other cases (24, 25) or that the soluble enzyme is more accessible to PQ-1.

**Purification**—The first purification step was ammonium sulfate fractionation. Although this procedure did not improve the purification factor (Table I), it separated the SQR from most of the carotenoids which remained in the 75% ammonium sulfate supernatant.

Attempts to further purify the enzyme by ion-exchange, dye resins, hydroxylapatite chromatography, or density gradients were unsatisfactory. Purification of the SQR was, however, obtained by gel filtration followed by hydrophobic chromatography and an additional gel filtration step (Table I, Figs. 1 and 2).

In the first gel filtration the proteins were resolved into three major peaks, the first two containing chromophores, as indicated by the 440 nm absorption (Fig. 1A). The first peak contained chlorophyll and no activity. The second broad peak was composed of two major components, the first one contained chlorophyll while the second contained carotenoids (not shown). The SQR activity peaked between these two components (Fig. 1A). The third peak did not contain a chromophore and was inactive. The overall purification in the pooled four most active fractions obtained by the first gel filtration was 3-fold (Table I).

The proteins of the pooled active fractions from the first gel filtration step are shown in Fig. 2A. Enrichment in high molecular weight polypeptides is observed. A 15-kDa polypeptide was most abundant in the former steps (P75 and DM sup) and comprised the non-active third major protein peak of the gel filtration (Fig. 1A).

The hydrophobic column further increased the purification by 5-fold (Table I), yielding two major polypeptide bands of 57 and 33 kDa (Fig. 2A). At this step the SQR fractions were practically free of chlorophyll but still contaminated by carotenoids (not shown). The following second gel-filtration step separated the SQR from this contamination. It yielded two peaks (detected at 280 and 440 nm): the first one contained the activity while the second the carotenoids (Fig. 1C, peaks 1 and 3, respectively). The estimated molecular mass of the active component was 75–85 kDa.

Fig. 2B shows the polypeptides resolved from the peak fractions of the second gel filtration, containing the SQR activity. A major single polypeptide of 57 kDa correlated with the level of activity. The apparent purification factor was 105 (Table I). Due to a partial loss of activity during purification, the purification factor is underestimated. The small difference in molecular weight between the denatured (Fig. 2B) and the active enzyme separated by gel filtration (Fig. 1C) can be ascribed to the DM molecules bound to the enzyme. Therefore, the SQR is most likely composed of a single polypeptide monomer.
Sulfide-Quinone Reductase from O. limnetica

In the purified SQR fraction (Fig. 2B, lanes c and d), a minor contamination of two polypeptides (33 and 35 kDa) was occasionally observed. The peaks of these polypeptides (Fig. 2B, lanes e and f and Fig. 1C, arrow 2) do not overlap either the 57-kDa or the SQR activity peaks. Analysis of the SQR peak purity of the fraction eluted from the last purification step (Fig. 1C, peak 1 at 32.67 min retention time, containing the polypeptides observed in Fig. 2B, lane d), by the D-6500 DAD (diode-array detector) System Manager software (Merck-Hitachi) indicates peak purity of 0.983.

The Prosthetic Group—The absorption spectra of the three distinct optical species separated by the last gel filtration step (Fig. 1C, peaks 1-3) are shown in Fig. 3. The major absorbing species (Fig. 3B, spectrum 3) was a carotenoid, with an absorption peak of ~458 nm. No polypeptide bands were detected for this species (Fig. 2B, lanes i and j). The least absorbing species (Fig. 1C, arrow 2) had maxima at 280 and 411 nm. The latter (on expanded scale) was characteristic of a Soret band of a cytochrome (not shown). This species coeluted with the polypeptide doublet of 33-35 kDa observed in Fig. 2B (lanes e and f).

The absorption spectrum of the purified enzyme showed a major band at 280 nm and two broad peaks at 367 and 460 nm (Fig. 3A) with a ratio A280/A460 of 9.2, typical of flavoproteins (26).

Flavins and flavoproteins exhibit characteristic fluorescence, with an emission peak around 520 nm. The fluorescence emission of the SQR peaked at 527 nm (Fig. 4A, inset). The excitation spectrum resembled the absorption spectrum, having two prominent peaks in the visible range (at 373 and 461 nm). In addition, there was only a very weak peak at 280 nm (Fig. 4). This may reflect energy transfer between a tryptophan and the flavin moiety, indicating a close proximity between them. Excitation spectra of spinach ferredoxin-NADP reductase (purchased from Sigma) assayed under similar conditions, resembled SQR in the visible range, but did not have the UV peak (not shown).

In some flavoproteins the more fluorescent form is the oxidized enzyme, while in others it is the reduced one (27). Fig. 4 shows that while SQR is fluorescent in its oxidized form, the fluorescence is fully quenched upon the addition of sulfide. Similarly, the absorption peaks of SQR at 367 and 460 nm were markedly reduced by either dithionite or sulfide (not shown). There was no effect of sulfide on the fluorescence spectra of ferredoxin-NADP reductase (not shown).

The spectral properties of SQR were found not to be affected by KCN (not shown).

The SQR Protein Is Induced by Sulfide—The SQR activity is known to be present only in membranes obtained from cells induced by sulfide (12) and de novo protein synthesis is required for this induction (5). Membranes prepared from cells incubated with sulfide in the presence of chloramphenicol do not exhibit SQR activity (12). To test whether the 57-kDa protein is only synthesized under inducing conditions, [35S]methionine was added to cells incubated anaerobically in the light, under both inducing and non-inducing conditions. Fig. 5A shows that a 57-kDa protein was strongly labelled under inducing conditions (lane 1, arrow 3). There was only a very weak labeling of a 57-kDa protein in the non-induced samples (lanes 2 and 4). Since the Coomassie staining showed a strong 57 kDa band under both induced and non-induced conditions (data not shown), we suggest that the protein labeled under the non-inducing conditions is a protein different from but comigrating with SQR. Fig. 5A also shows that the minor contaminants of 33 and 35 kDa present in some fractions of the SQR preparations (Fig. 2B) are not inducible by sulfide. They are therefore not present only in membranes obtained from cells induced by sulfide (12) and de novo protein synthesis is required for this induction (5). Membranes prepared from cells incubated with sulfide in the presence of chloramphenicol do not exhibit SQR activity (12). To test whether the 57-kDa protein is only synthesized under inducing conditions, [35S]methionine was added to cells incubated anaerobically in the light, under both inducing and non-inducing conditions. Fig. 5A shows that a 57-kDa protein was strongly labelled under inducing conditions (lane 1, arrow 3). There was only a very weak labeling of a 57-kDa protein in the non-induced samples (lanes 2 and 4). 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related to the SQR protein complex, nor are they a degradation product of the 57-kDa protein.

A polyclonal antibody raised against the 57 kDa band was used to probe preparations from sulfide-induced and non-induced cells. The antibody recognized specifically a 57-kDa protein found only in the induced membranes or their DM extract (Fig. 5B, lanes 1 and 3). To exclude the possibility that the minor impurities which occasionally copurify with SQR, 33- and 35-kDa polypeptides (see Fig. 2B) might be related to SQR, we raised another polyclonal antibody, which recognized the 33 and 35 polypeptides, but not the 57-kDa polypeptide. Using this antibody, we found that whereas the 57-kDa polypeptide is induced by sulfide (Fig. 5B), the short polypeptides were not induced (data not shown). This immunological data and the peak purity analysis of the purified SQR (see above) jointly imply that the 57-kDa protein is the SQR.

Properties of the Purified SQR—The concentration dependence of the purified SQR on its substrates PQ-1 and sulfide was determined (Fig. 6). The apparent $K_m$ values (31 and 8 µM, respectively) were very similar to those of the SQR activity in isolated membranes (12).

The inhibitor sensitivity of SQR activity in its isolated form, as compared with the membrane-bound SQR, and with the SQR-dependent NADP photoreduction in the membranes, is demonstrated in Table II. Aurachin C and NQNO are quinone-analogs which inhibit the $b_{6f}/b_{c1}$ complexes at the Qc site (29).

Both analogs inhibited all the above three reactions with aurachin being the most potent inhibitor of the soluble SQR. The $I_{50}$ (7 nm) is the lowest reported for this inhibitor so far. However, DNP-INT, a typical inhibitor of the Qs site in chloroplasts (30), inhibited only the full electron transport reaction to NADP but not the direct SQR reaction of both the membrane-bound and isolated enzyme (Table I). All three reactions were insensitive to the $Q_b$ inhibitor 3-(3',4'-dichlorophenyl)-1,1-dimethylurea. The results demonstrate that the isolated SQR maintains the same selective sensitivity to quinone analog inhibitors as the membrane-bound enzyme (Table II and Ref. (12)). KCN, a potent inhibitor of flavoproteins, also inhibited the isolated SQR.

Based on the inhibition of the membranal SQR by Qc inhibitors we proposed two alternative models for the functional location of SQR in thylakoids (12). In one model the SQR formed a super complex with the cytochrome $b_{6f}$ complex, sharing the Qc quinone-binding site with cytochrome $b_{6f}$. The alternative model put the SQR as an independent enzyme, transferring electrons to the PQ pool via its own quinone-binding site, which we named Qs. The results presented here, showing that the SQR can be isolated out of the membrane as an active single polypeptide which maintains its typical differential inhibitor sensitivity, strongly suggest that the second model is the correct one. Thus, the differential sensitivity reflects the affinity of the Qs site to quinone analogs. We propose that the enzyme contains binding sites for sulfide and for quinone and that the electron transfer between them is mediated by the flavin cofactor. According to the EC nomenclature, the SQR should be defined as EC 1.8.5.7. It was previously suggested that in several species of photo-
Sulfide-Quinone Reductase from O. limnetica

![Graph](image)

**Fig. 6.** Substrates concentration dependence of the purified SQR. A, PQ-1 concentration dependence. Sulfide concentration was maintained constant at 110 μM. B, dependence on sulfide. PQ-1 concentration was maintained constant at 40 μM. Rates were corrected for the non-catalytic reaction.

**Table II**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Known target sites</th>
<th>H2→NADP membrane</th>
<th>SQ membrane</th>
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**TABLE II**

Inhibitors of the membrane-bound and isolated O. limnetica SQR

I50 is the inhibitor concentration required for 50% inhibition of the activity. DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea.

There are three prokaryotes, test the R. capsule transform into soluble cytochrome c. However, the role of flavocytochrome c as the major initial step in sulfide oxidation has recently been questioned because it is absent from several sulfide-oxidizing bacteria (31, 32). Alternatively, the transfer of electrons from sulfide primarily into the quinone pool was proposed (31, 34).

We have recently purified the SQR also occurs in membranes of the photosynthetic green bacterium Chlorobium limicola f. thiosulfatophilum and the purple bacterium Rhodobacter capsulatus (35, 36). Of the three prokaryotes tested, the R. capsulatus enzyme has the highest affinity to sulfide (apparent \( K_m \) = 2 μM). Unlike O. limnetica, in both Chlorobium and R. capsulatus, the enzyme is constitutive in cells grown in the absence of sulfide. The quinone pool of these prokaryotes is each composed of a different quinone species: PQ-9 in Oscillatioria, MQ-7 and Chromobium-quinone in Chromobium, and UQ-10 in R. capsulatus. Therefore, the quinone-binding site (QS) of each SQR species is expected to differ, accordingly. This is indeed reflected by the different pattern of sensitivity to quinone-analog inhibitors (36).

The Amino Acid Sequence of the Amino Terminal of the Purified SQR—The sequence of the first 29 amino acids of the NADH terminus of the O. limnetica purified SQR is shown in Fig. 7. Remarkably, right from the second residue, this sequence contains the highly conserved fingerprint of the ADP-binding site of many NAD/FAD-binding proteins (37–39). This ADP binding site can be recognized by a common fingerprint which consists of a set of 11 rules describing the type of amino acids that should occur at a specific position in a peptide fragment. The total length of this fingerprint varies between 29 and 31 residues. While the 3 glycine residues as well as the acid residues at the end of the fingerprint are strictly conserved, the others can be changed according to the rules (37). The O. limnetica NADH terminus contains the 3 conserved glycines as well as 6 additional residues of the fingerprint in the right spacing (Fig. 7). The last 2 expected residues are most probably located beyond the sequenced area. Unlike the NAD-binding domains, the FAD-binding domains of many flavoenzymes appear to be located close to the amino terminus (38, 39). These results, therefore, suggest that the prosthetic group of SQR is FAD.

We have recently purified the SQR of R. capsulatus and sequenced 18 amino acids of its NADH terminus (Fig. 7). Although the sequenced area is short, it shows a high homology to O. limnetica NADH terminus (61% identity (16/18) and 89% similarity (15/18)). Accordingly, starting from the second amino acid, all the expected fingerprint residues of the NAD/FAD-binding domain exist in the R. capsulatus NH2 terminus. We therefore propose that the SQR is a universal flavoenzyme which plays a major role in sulfide-dependent anoxygenic photosynthesis in prokaryotes.

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edged for sequencing the *O. limnetica* and the *R. capsulatus* SQR N termini, respectively.

REFERENCES