

Phyton (Horn, Austria) Special issue: "Sulfur-Metabolism"	Vol. 32	Fasc. 3	(133)-(137)	18. 12. 1992
---	---------	---------	-------------	--------------

Isolation of Sulfide-Quinone Reductase (SQR) from Prokaryotes

By

Y. SHAHAK¹⁾ B. ARIELI²⁾, G. HAUSKA³⁾, I. HERRMANN³⁾ & E. PADAN²⁾

Key words: *Chlorobium limicola f. thiosulfatophilum*, *Oscillatoria limnetica*, *Rhodobacter capsulatus*, anoxygenic photosynthesis, cyanobacteria, quinone-binding protein, sulfide oxidation.

Summary

SHAKAK Y., ARIELI B., HAUSKA G., HERRMANN I. & PADAN E. 1992. Isolation of sulfide-quinone reductase (SQR) from prokaryotes. - *Phyton* (Horn, Austria) 32 (3): (133)-(137).

Sulfide-quinone reductase (SQR) is a membranal electron carrier which catalyzes the initial step in sulfide-dependent anoxygenic photosynthesis in prokaryotes. We report here on the isolated and partially purified SQR of the cyanobacterium *Oscillatoria limnetica*. The functional enzyme is a quinone-binding protein which is probably composed of a single polypeptide, having an apparent molecular weight (on SDS-PAGE) of 57 KDa. SQR was also isolated from the bacteria *Rhodobacter capsulatus* and *Chlorobium limicola f. thiosulfatophilum*.

Introduction

Oscillatoria limnetica is a filamentous cyanobacterium which can switch from oxygenic (plant-type) to anoxygenic (bacterial-type) photosynthesis (PADAN 1979). The switch to the anoxygenic mode is induced by sulfide under light and anaerobic conditions, and depends on the synthesis of new proteins (see BELKIN & al. 1987, for review). Thylakoids prepared from induced cells maintain the induced capacity to catalyze sulfide-dependent NADP-photoreduction. Like the anoxygenic reaction in whole cells, the cell-free system photoreaction is sensitive to all inhibi-

¹⁾ Institute of Horticulture, The Volcani Center, P.O.B. 6 Bet-Dagan 50250, Israel.

²⁾ Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem 91904, Israel.

³⁾ Institut für Zellbiologie und Pflanzenphysiologie, Universität Regensburg, 8400 Regensburg, Germany.

tors of the cytochrome *b₆f* complex, but insensitive to DCMU, an inhibitor of the photosystem II QB site (SHAHAK & al. 1987). Assay conditions were developed for a partial dark electron transfer reaction from sulfide to externally added quinones. It enabled us to define the inducible enzyme which catalyzes the initial step in anoxygenic photosynthesis as sulfide-quinone reductase (SQR), a novel photosynthetic electron carrier (ARIELI & al. 1991).

Materials and Methods

Cyanobacterial growth, sulfide-induction, thylakoid preparation and the assay conditions for SQR activity and for sulfide-dependent NADP photoreduction were described in ARIELI & al. 1991.

Abbreviations: SQR, Sulfide-quinone reductase; PQ, plastoquinone; UQ, ubiquinone; NQNO, 2-*n*-nonyl-4-hydroxyquinoline-N-oxide; DNP-INT, 2-iodo-6-isopropyl-3-methyl-2'-4,4'-trinitrophenyl ether; DCMU, 3-(3,4'-dichlorophenyl)-1,1-dimethylurea; DM, *n*-dodecyl β -D-mal-
toside.

Results and Discussion

The treatment of induced *O. limnetica* thylakoids (thoroughly washed in 5 mM EDTA to release peripheral proteins) with detergents resulted in the release of SQR activity from the membranes. Similar treatment of non-induced thylakoids yielded no SQR activity at all. Mild treatment with a combination of 25 mM dodecyl-maltoside and 0.4 % cholate-Na was sufficient to solubilize most of the inducible membranal enzyme in an active and stable form. The purification steps included ammonium sulfate fractionation (55-75 %), followed by several HPLC steps (gel filtration, hydrophobic chromatography and a second gel filtration. DM (5 mM) was present throughout the procedure (ARIELI & al., in preparation). In Fig. 1 the elution profile of the final step is demonstrated. SQR activity was eluted as a sharp peak corresponding to about 67 to 80 KDa. It was not fully separated from an orange contaminant of about 40 KDa, which is probably a carotenoid protein (REDDY & al. 1992). However, SDS-PAGE of the SQR active fractions, stained in either Commassie-blue or silver, clearly correlated the SQR with one major band (over 95 % of the total stained bands) of 57 KDa (apparent MW). The "carotenoid-protein" minor contaminant ran as a 35 KDa polypeptide on the denaturing gel. We therefore conclude that the functional form of SQR is composed of a single polypeptide. In solution it formed mixed-micelles with DM molecules, accounting for the higher MW in gel filtration.

The inhibitor sensitivity of the cyanobacterial SQR activity in its isolated form, as compared with the membrane-bound SQR, and with NADP photoreduction in the membranes, which is SQR-dependent, is demonstrated in Table 1. Aurachin C and NQNO are quinone-analogs which inhibit the *b₆fbc₁* complexes at the Qc site (OETTMEIER & al. 1990). Both analogs inhibited all the above three re-

actions, aurachin being an extremely potent inhibitor of the soluble SQR. However DNP-INT, a typical inhibitor of the Qz site in chloroplasts (HAEHNEL & TREBST 1982) inhibited only the full electron transport reaction to NADP, but not the direct SQR reaction of both membrane-bound and isolated enzyme (Table 1). All three reactions were insensitive to QB inhibitors. The results demonstrated that the isolated SQR maintains the same selective sensitivity to quinone analog inhibitors as the membrane-bound enzyme.

Table 1. Sensitivity of membrane-bound and isolated *O. limnetica* SQR to quinone-analog inhibitors. I_{50} is the inhibitor concentration required for 50 % inhibition of the initial rate of either sulfide-dependent NADP photoreduction ($H_2S \rightarrow NADP$) or sulfide-dependent reduction of PQ^{-1} (SQR).

Inhibitor		I_{50} (nM)		
Name	Known target sites	$H_2S \rightarrow NADP$ memb.	SQR memb.	SQR isolated
Aurachin C	Qc and QB	39	49	7
NQNO	Qc	150	140	72
DNP-INT	Qz	1350	none	none
DCMU	QB	none	none	none

In ARIELI & al. 1991, we proposed two alternative models for the functional location of SQR in thylakoids. In one model the SQR formed a super-complex with the cytochrome *b₆f* complex, sharing the Qc quinone binding site with cytochrome *b₆*. The alternative model put the SQR as an independent enzyme, transferring electrons to the PQ pool via a quinone binding site (which we named Qs) on the SQR. The results presented here, showing that 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 (Fig. 3) is the correct one. Thus, the differential sensitivity reflects the affinity of the Qs site to quinone analogs. The SQR is, therefore, the smallest and most simple quinone binding enzyme described so far. The other, well known ones, form multisubunit complexes.

We have reported that SQR occurs also in membranes of the photosynthetic green bacterium *Chlorobium limicola* f. *thiosulfatophilum*, and suggested the universality of SQR as the enzyme catalyzing sulfide-quinone oxidoreduction in photoautotrophs (SHAHAK & al. 1992).

Indeed, we have recently detected SQR activity in chromatophores of an additional prokaryote, the purple bacterium *Rh. capsulatus*. SQR activity, measured with decyl-UQ as the electron acceptor, was highly sensitive to both aurachin and stigmatellin, and sensitive to NQNO. The high potency of stigmatellin occurred also in *Chlorobium* (SHAHAK & al. 1992), but not in *O. limnetica* (ARIELI & al. 1991). All three systems are sensitive to KCN ($I_{50} \approx 10 \mu M$), suggesting the content of a prothetic group in the three SQRs.

Based on its extraction by detergents, we proposed in Fig. 2 that the cyanobacterial SQR is embedded in the membrane. We do not have indications yet about its orientation in the membrane. The *Rh. capsulatus* SQR, however, can be washed out of the membrane by 2 M NaBr. This results may suggest that in this system the SQR is a rather peripheral enzyme.

Acknowledgements

This work was supported by the German-Israeli Foundation for Scientific Research and Development (GIF), Grant I-91-118. 9/88 (to E.P., Y.S. and G.H.) and by the Israeli Ministry of Science and Development, The National Council for Research and Development (to B.A.).

References

- ARIELI B., PADAN E. & SHAHAK Y. 1991. - J. Biol. Chem. 266: 104-111.
BELKIN S., SHAHAK Y. & PADAN E. 1987. - Methods Enzymol. 167: 380-386.
HAEHNEL W. & TREBST A. 1982. - J. Bioenerg. Biomembr. 14: 181-190.
OETTMEIER W., DOSTANTI R., MAJEWSKI C., HÖFLE G., FECKER T., KUNZE B. & REICHENBACH H. 1990. - Z. Naturforsch 45C: 311-328.
PADAN E. 1979. - Annu. Rev. Plant Physiol. 30: 27-40.
REDDY K. J., BULLERJAHN G. S. & SHERMAN L. A. 1992. - Methods Enzymol. (in press).
SHAHAK Y., ARIELI B., BINDER B. & PADAN E. 1987. - Arch. Biochem. Biophys. 259: 605-615.
— , — , PADAN E. & HAUSKA G. 1992. - FEBS Lett 299: 127-130.

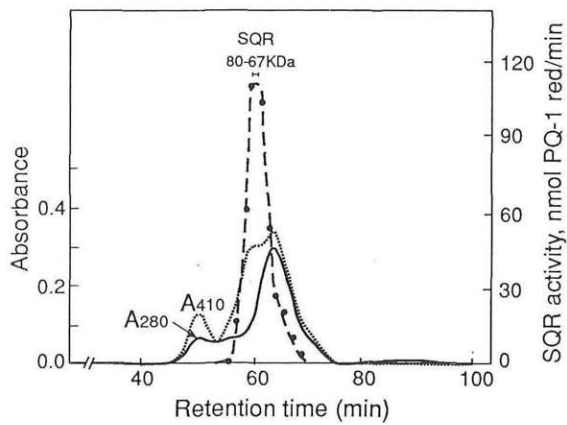


Fig. 1. Elution profile of isolated *O. limnetica* SQR preparation from gel filtration HPLC. See text for details.

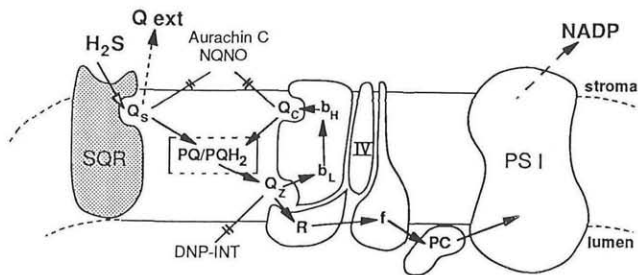


Fig. 2. Model for the location of SQR in thylakoids, Q ext. externally added quinone.

ZOBODAT - www.zobodat.at

Zoologisch-Botanische Datenbank/Zoological-Botanical Database

Digitale Literatur/Digital Literature

Zeitschrift/Journal: [Phyton, Annales Rei Botanicae, Horn](#)

Jahr/Year: 1992

Band/Volume: [32_3](#)

Autor(en)/Author(s): Shahak Y., Arieli B., Hauska Günter, Herrmann E., Padan E.

Artikel/Article: [Isolation of Sulfide-Quinone Reductase \(SQR\) from Prokaryotes. 133-137](#)