Volume 299, number 2, 127–130 FEBS 10795 March 1992

Sulfide quinone reductase (SQR) activity in *Chlorobium*

Y. Shahak[a], B. Arieli[b,*], E. Padan[b] and G. Hauska[c]

[a]*Biochemistry Department, The Weizmann Institute of Science, Rehovot 76100, Israel,* [b]*Division of Microbial and Molecular Ecology, Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem 91904, Israel* and [c]*Institut für Zellbiologie und Pflanzenphysiologie, Universität Regensburg, 8400 Regensburg, Germany*

Received 20 January 1992

Membranes of the green sulfur bacterium, *Chlorobium limicola* f. *thiosulfatophilum*, catalyze the reduction of externally added isoprenoid quinones by sulfide. This activity is highly sensitive to stigmatellin and aurachins. It is also inhibited by 2-*n*-nonyl-4-hydroxyquinoline-*N*-oxide, antimycin, myxothiazol and cyanide. It is concluded that in sulfide oxidizing bacteria like *Chlorobium*, sulfide oxidation involves a sulfide-quinone reductase (SQR) similar to the one found in *Oscilatoria limnetica* [Arieli, B., Padan, E. and Shahak, Y. (1991) J. Biol. Chem. 266, 104–111].

Sulfide oxidation; Quinone reduction; Electron transport; Photosynthesis; *Chlorobium limicola*

1. INTRODUCTION

The *Chlorobiaceae* are obligate photoautotrophic green bacteria, able to use H_2S and S°. *Chlorobium limicola* f. *thiosulfatophilum* can also utilize thiosulfate ($S_2O_3^{2-}$). The intermediates observed during H_2S oxidation by *Chlorobium* are thiosulfate and S°, the final oxidation product being sulfate (SO_4^{2-}) (see [1] for a review).

The initial step in sulfide electron donation into the photosynthetic electron transport chain is a matter of debate. In several species of phototrophic bacteria, the sulfide-oxidizing enzyme was proposed to be flavocytochrome *c*. Indeed, flavocytochrome *c*, which has been found and isolated from several sulfur bacteria ([2], see also [1]), catalyzes electron transfer from H_2S to 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 ([3], see also [1]). Alternatively, the transfer of electrons from sulfide primarily into the quinone pool was proposed [1,4].

Sulfide-dependent anoxygenic photosynthesis occurs also in several species of cyanobacteria. *Oscillatoria limnetica*, which is the most thoroughly studied species, can readily shift from oxygenic (plant-type) to anoxygenic photosynthesis. The shift is induced by sulfide under light and anaerobic conditions, and depends on the synthesis of new proteins [5,6]. Sulfide-dependent CO_2 fixation involves the cytochrome b_6f complex and photosystem I, but not photosystem II [6–8]. Light-dependent sulfide oxidation is maintained in isolated, washed thylakoids prepared from induced cells [9]. Recently we have found that the induced thylakoids can specifically catalyze sulfide-dependent reduction of externally added quinones, in the dark. We proposed that a novel electron carrier, sulfide-quinone reductase (SQR), is the inducible protein catalyzing the initial step in sulfide oxidation in *O. limnetica* [10]. The SQR was further solubilized from induced *O. limnetica* thylakoids, and purified. The isolated enzyme maintains its high affinity to sulfide and quinone, as well as its differential sensitivity to inhibitors (Arieli, Shahak and Padan, in preparation).

In the present work we report on the occurrence of SQR also in *Chlorobium* and suggest the universality of SQR as the enzyme catalyzing sulfide-quinone oxidoreduction in photoautotrophs.

Correspondence address: Y. Shahak, Institute of Horticulture, The Volcani Center, PO Box 6, Bet-Dagan 50-250, Israel.

**Present address:* Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, USA.

Abbreviations: SQR, sulfide-quinone reductase; MQ, menaquinone; PQ-1(-2), plastoquinone-1(-2); UQ-1(-2), ubiquinone-1(-2); NQNO, 2-*n*-nonyl-4-hydroxyquinoline-*N*-oxide; UHDBT, 5-*n*-undecyl-6-hydroxy-4,7-dioxobenzothiazole; DNP-INT, 2-iodo-6-isopropyl-3-methyl-2′-4,4′-trinitrodiphenyl ether; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; Bchl *c*, bacteriochlorophyll *c*.

2. MATERIALS AND METHODS

Cells from *Chlorobium limicola* f. *thiosulfatophilum* were grown and membranes were prepared as described before [11]. Membranes were stored at −20°C. Bacteriochlorophyll *c* concentration was determined from the spectra in aqueous solution, using an extinction coefficient of 90 for 1 mg Bchl *c*/ml at 750 nm, which was estimated from a parallel measurement in methanol [12].

Spectra were measured and processed by a Kontron Uvikon 860 spectrophotometer equipped with a stirred and thermostated cuvette holder, which was placed close to the detector to minimize light scat-

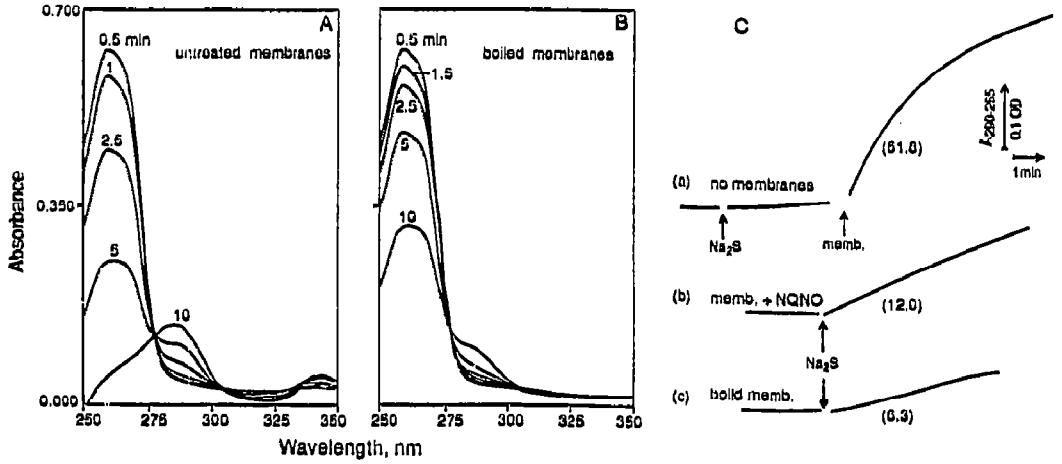

Fig. 1. Sulfide-dependent plastoquinone-1 reduction catalyzed by *Chlorobium* membranes. Reaction mixture contained 20 mM TRIS-HCl, pH 7.8, membranes containing 8.4 μg Bchl *c*/ml and either 40 μM PQ-1 and 200 μM Na_2S (A and B) or 20 μM PQ-1 and 40 μM Na_2S (C). (A) and (B) illustrate spectra scanned at the indicated times after PQ-1 injection, after the digital subtraction of the spectrum taken shortly prior to the injection. Each scan took 15 s for completion. The indicated times are the starting times. In (B) the membranes were incubated for 10 min at 100°C before addition. (C) illustrates continuous monitoring of PQ-1 reduction by dual wavelength spectroscopy. Where indicated, 4 μM NQNO was added to the membranes. For other details see Materials and Methods. The numbers in parentheses in Fig. 1C indicate the initial quinone reduction in μmol/mg Bchl *c*·h.

tering effects. Dual-wavelength absorption changes were monitored continuously by an Aminco DW-2a spectrophotometer as previously described [10].

The reactions were carried out under N_2 atmosphere in stoppered quartz cuvettes at 24°C. Typically, the reaction mixture (3 ml) was composed of 20 mM TRIS-HCl, pH 7.8, 40 μM quinone, 40 μM Na_2S, and membranes containing 8–10 μg Bchl *c*/ml.

Preparation of stock solutions and concentration determination of quinones and quinone-analog inhibitors was done as described in [10]. PQ-1 was synthesized according to Rich [13]. PQ-2, UQ-1 and UQ-2 were gifts of Dr. G. Hind, Brookhaven National Laboratory, USA, of Hoffmann-La Roche, Switzerland and of Dr. E. Keynan, The Technion, Israel, respectively. Duroquinone was purchased from Aldrich, antimycin A, vitamin K_1 and DCMU from Sigma. The following quinone-analog inhibitors were generously donated: stigmatellin, and aurachin C and D by Dr. G.H. Höfle, Braunschweig, Germany; NQNO and UHDBT by Dr. B.L. Trumpower, Dartmouth Medical School, Hanover, USA; and DNP-INT by Dr. A. Trebst, Ruhr University, Bochum, Germany.

3. RESULTS AND DISCUSSION

Chlorobium membranes catalyze the electron transfer from sulfide to externally added quinones, in the dark. Fig. 1 illustrates sulfide-dependent PQ-1 reduction. The spectral changes indicate that PQ-1 became fully reduced within less than 10 min of incubation with sulfide and *Chlorobium* membranes, under the experimental conditions (Fig. 1A). Fig. 1B shows that with boiled membranes, the spectral changes developed much slower. The rate of the enzymatic reaction, unlike the boiled control, decreased with time (compare Fig. 1C, traces (a) and (c)). NQNO, a quinone analog which has previously been found to specifically inhibit SQR activity in *O. limnetica* [10], also inhibited it in *Chlorobium* (Fig. 1C, trace (b)), further supporting the enzymatic nature of the reaction. NQNO had no effect on the rate with boiled membranes (not shown).

In addition to the enzymatic reaction, *Chlorobium* membranes seem to contain an unknown, heat-stable substance which can stimulate sulfide-quinone oxidoreduction. The initial rates of quinone reduction by sulfide, measured in the presence of boiled membranes, was markedly faster than that in the absence of membranes. For example, the ratio of the rate with boiled membranes to the one in absence of membranes in Fig. 1C is about 4 (traces (a) and (c)). This ratio further increased upon increasing the substrate concentrations. In accordance, also the NQNO-insensitive portion of quinone reduction by sulfide in intact membranes was much more pronounced at high substrate concentrations. These background rates did not decrease with time, but the enzymic reaction did (Fig. 1C, trace (a)). Furthermore, at higher sulfide concentrations (>200 μM) the reduction of quinone in the absence of membranes increased substantially within the measuring time of a few minutes. Probably, chemical reduction of quinones is autocatalytically stimulated by the accumulation of small amounts of semiquinone during the course of the reaction [14]. Therefore, we avoided using sulfide and quinone concentrations higher than 40 μM, and we corrected SQR rates for the rates obtained with boiled membranes. It is worth mentioning that in *O. limnetica* the initial rates of sulfide-dependent PQ-1 (or PQ-2) reduction measured in the absence of membranes or with boiled membranes are comparable [10].

In the experiments summarized in Table I we have tested several externally added quinones for their activity with *Chlorobium* SQR. Ubiquinone and plastoqui-

Table I

Chlorobium SQR activity with different quinone acceptors

Quinone acceptor	SQR initial rate (μmol Q red/mg Bchl c·h)
Vitamin K_1	0
Menadione	1.9
2-methyl-3-methylthio-1,4-naphto-quinone	0.8
UQ-1	5.0
UQ-2	15.9
PQ-1	17.5
PQ-2	27.4
Duroquinone	1.3

The reaction mixture was as described for Fig. 1, with quinones present at 20 μM. Sulfide concentration was 56 μm. The following coefficients were used (for 1 mM): 14.8 for vitamin K_1, menadione and methylthio menadione (at 295–270 nm); 16.9 for PQ-1 and PQ-2 (at 292–266 nm); 17.8 for UQ-1 or UQ-2 (at 300–280 nm); and 18.0 for duroquinone (at 295–272 nm; see [10]). Rates were corrected for controls with boiled membranes.

none, which have short isoprenoid side chains, were good external acceptors. In both cases a side chain composed of two isoprenoid units was preferable to one unit. Duroquinone, which lacks an extended side chain, was a relatively poor acceptor. Since the endogenous quinone pool in *Chlorobium* consists of MQ-7 and 1'-oxo-MQ-7 (*Chlorobium* quinone; see [15]), we also tested 3 naphtoquinones. However, they gave no, or only poor, reduction rates (Table I). Menadione and 2-methyl-3-methylthio-1,4-naphtoquinone, like duroquinone, lack an extended side chain, and vitamin K1 (phylloquinone) may be too insoluble to partition fast enough into the membranes. Alternatively, if *Chlorobium* quinone, with its high redox potential [15] functions as the physiological electron acceptor, the redox potential of 2,3-substituted 1,4-naphtoquinones may be too low [15,16], and the ones of PQ and UQ may just suffice [17,18]. It is worth mentioning in this context that electron flow from succinate to MQ is energy-dependent in bacilli [19], and therefore is attenuated in non-vesicular, cell-free systems.

Table II

Inhibitors of SQR activity in *Chlorobium*

Inhibitor	I_{50} (nM)
Stigmatellin	5
Aurachin C	12
Aurachin D	38
NQNO	760
Antimycin A	960
Myxothiazol	6,000
KCN	10,000
UHDBT	No effect
DNP-INT	No effect
DCMU	No effect

Reaction conditions were as for Fig. 1C. I_{50} is the inhibitor concentration required for 50% inhibition of SQR initial rate. The inhibitors were added to the reaction mixture containing the membranes (while stirring), 1–2 min prior to the sequential addition of PQ-1 and Na_2S.

The *Chlorobium* SQR activity was extremely sensitive to three naturally occurring inhibitors (Table II): stigmatellin, and aurachin C and D. They inhibit electron transfer at the Q_B site of photosystem II and at the cytochrome b_6f complex, as well as at the mitochondrial and bacterial cytochrome bc_1 complex [20–22]. Under our experimental conditions, the *Chlorobium* SQR activity was sensitive to nanomolar concentrations of the inhibitors, with stigmatellin the most potent (I_{50} = 5 nM; pI_{50} = 8.3). No other system studied so far is as sensitive to stigmatellin. NQNO and the two other antibiotics, antimycin and myxothiazol, inhibited at μM concentrations.

It is of interest to compare the inhibitor sensitivity of *Chlorobium* SQR with the *O. limnetica* SQR. The aurachins inhibit the cyanobacterial enzyme equally well (Arieli, Shahak and Padan, in preparation), but stigmatellin is by far a superior inhibitor of the *Chlorobium* enzyme. Unlike in *Chlorobium*, in *O. limnetica* the SQR is not inhibited at all by either antimycin or myxothiazol [10]. Also KCN only inhibits the *Chlorobium* SQR. The opposite is true for UHDBT, which is a fairly good inhibitor in *O. limnetica*. SQR in both systems is insensitive to DNP-INT or DCMU. The differences between the two systems might reflect differences in the quinone binding site of each enzyme, which might be related to the different endogenous quinone acceptor in each system.

The observed rates of SQR in *Chlorobium* membranes and the rates reported for the reduction of cytochrome *c* by sulfide via flavocytochrome *c*-553 of *Chlorobium* are comparable, corresponding to some 10 s^{-1} in TON. Since the flavocytochrome is largely washed out from our membranes we conclude that sulfide oxidation in *Chlorobium* is not exclusively occurring via flavocytochrome *c*, but also via SQR and the quinone pool [1,4], as in *Oscillatoria*. This path may be more tightly linked to energy conservation.

Acknowledgements: Dr. A. Trebst, Bochum, and Dr. H.G. Trüper, Bonn, are highly acknowledged for helpful discussions, the latter also for handling the transportation of frozen *Chlorobium* membranes from Germany to Israel. This work was supported by the German–Israel Foundation for Scientific Research and Development (GIF), Grant I-91-118.9/88.

REFERENCES

[1] Brune, D.C. (1989) Biochim. Biophys. Acta 975, 189–221.
[2] Kusai, A. and Yamanaka, T. (1973) Biochim. Biophys. Acta 304, 304–314.
[3] Steinmetz, M.A., Trüper, H.G. and Fischer, U. (1983) Arch. Microbiol. 135, 186–190.
[4] Trumpower, B.L. (1990) Microbiol. Rev. 54, 101–129.
[5] Oren, A. and Padan, E. (1978) J. Bacteriol. 133, 558–563.
[6] Padan, E. (1979) Annu. Rev. Plant Physiol. 30, 27–40.

[7] Oren, A., Padan, E. and Avron, M. (1977) Proc. Natl. Acad. Sci. USA 74, 2152–2155.
[8] Belkin, S., Shahak, Y. and Padan, E. (1987) Methods Enzymol. 167, 380–386.
[9] Shahak, Y., Arieli, B., Binder, A. and Padan, E. (1987) Arch. Biochem. Biophys. 259, 605–615.
[10] Arieli, B., Padan, E. and Shahak, Y. (1991) J. Biol. Chem. 266, 104–111.
[11] Nitschke, W., Feiler, U., Lockau, W. and Hauska, G. (1987) FEBS Lett. 218, 283–286.
[12] Stanier, R.W. and Smith, J.H.C. (1960) Biochim. Biophys. Acta 41, 478–484.
[13] Rich, P.R., Heathcote, P. and Moss, D.A. (1987) Biochim. Biophys. Acta 892, 138–151.
[14] Michaelis, L., Schuber, M.P., Reber, R.K., Kuck, J.A. and Granick, S. (1938) J. Am. Chem. Soc. 60, 1678–1683.
[15] Powls, R. and Redfearn, E.R. (1969) Biochim. Biophys. Acta 172, 429–437.
[16] Clark, W.M. (1960) Oxidation and Reduction Potentials of Organic Systems, Williams and Wilkins, Baltimore.
[17] Urban, P.F. and Klingenberg, M. (1969) Eur. J. Biochem. 9, 519–525.
[18] Golbeck, J.H. and Kok, B. (1979) Biochim. Biophys. Acta 547, 347–360.
[19] Lemma, E., Unden, G. and Kröger, A. (1990) Arch. Microbiol. 155, 62–67.
[20] Oettmeier, W., Godde, D., Kunze, B. and Hoefle, G. (1985) Biochim. Biophys. Acta 807, 216–219.
[21] Oettmeier, W., Dostatni, R., Majewski, C., Hoefle, G., Fecker, T., Kunze, B. and Reichenbach, H. (1990) Z. Naturforsch. 45c, 311–328.
[22] Thierbach, G., Kunze, B., Reichenbach, H. and Hoefle, G. (1984) Biochim. Biophys. Acta 765, 227–235.