Do Cyanobacteria Contain a Membrane Bound Cysteine Oxidase?

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The oxidation of cysteine with O_2 is facilitated by isolated membranes of the cyanobacteria *Anacystis nidulans* and *Anabaena variabilis*, and further stimulated by Fe^{3+}. This reaction accelerates with increasing the pH value and is suppressed by cyanide, benzylhydroxamate, hydroxylamine, but not azide. The agents mentioned inhibited the respiration of the membranes with ascorbate and N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD) effectively, but did not influence their nonenzymic oxidation. It is inferred that ascorbate in combination with TMPD is oxidized via membrane oxidases. Cysteine oxidation apparently proceeds non-enzymatically and is catalyzed by the cations of heavy metals.

Introduction

Cyanobacteria are known to posses a respiratory electron transfer chain in addition to the photosynthetic one. Cytochromes aa_3 [1–4] and a CN^--resistant, benzylhydroxamate-sensitive oxidase of alternative type [5] function as terminal oxidase in the respiratory chain of a number of cyanobacteria. Respiration of cyanobacteria is inhibited by CO, CN^-, N_3^-, NH_2OH, the derivatives of hydroxamic acid and other agents. NADH, NADPH, H_2, succinate, ascorbate in combination with TMPD [3–5] are suitable as oxidizable substrates.

Schmidt and Krämer [6] presented data on the CN^- sensitive cysteine oxidation in the suspension of isolated cyanobacterial membranes. The oxidation of four cysteine molecules was accompanied by the uptake of one O_2 molecule. The authors concluded that cysteine is oxidized with the participation of the cytochrome oxidase [6].

Data on cysteine oxidation stimulated by Fe^{3+} and inhibited by CN^-, benzylhydroxamate, NH_2OH are reported in this paper. The results obtained question the validity of the possibility of enzymic oxidation of cysteine in cyanobacterial respiratory chain.

Materials and Methods

Cells of *Anacystis nidulans* and *Anabaena variabilis* Kütz were cultivated in the 750-ml flasks in the medium "C" [7] at 26–28 °C under the white light intensity of 1000 lux. The cells from 5 to 7-day cultures corresponding to the late exponential phase of growth were washed twice with 20 mM HEPES/NaOH buffer (pH 7.0) and suspended in the same buffer. *A. variabilis* cells were disrupted at 0 °C by sonication twice for 15 s at 22 kHz and *A. nidulans* cells were sonicated 4 times for 30 s with 60 s interruptions. The cell homogenates were centrifugated at $8000 \times g$ for 15 min to remove the unbroken cells and the cell walls. The supernatant was centrifuged at 2–4 °C for 60 min at $105\,000 \times g$. The sediment of the membranes was washed and suspended in 20 mM HEPES/NaOH buffer solution (pH 7.5) and was kept in a concentrated state at 4 °C in the dark. The chlorophyll content was estimated using the extinction coefficient of $60\ mM^{-1} \times cm^{-1}$ at 675 nm [8]. O_2 uptake was monitored polarographically with a Clark-type electrode. The experimental conditions are given in the figure and table captions. L-cysteine was obtained from GIBCO (NY., USA), KCN and NaN_3 from BDH (Poole, England), Tris, MES, HEPES, ascorbic acid and TMPD from Serva (Heidelberg, Germany). All chemicals were of maximum purity.

Results and Discussion

The addition of cysteine to the buffer solution caused an O_2 uptake (Fig. 1 A and B). The membranes of *A. nidulans* stimulated the O_2 uptake by a factor of 2–2.5 after lag-phase of 4–5 min. CN^- at a

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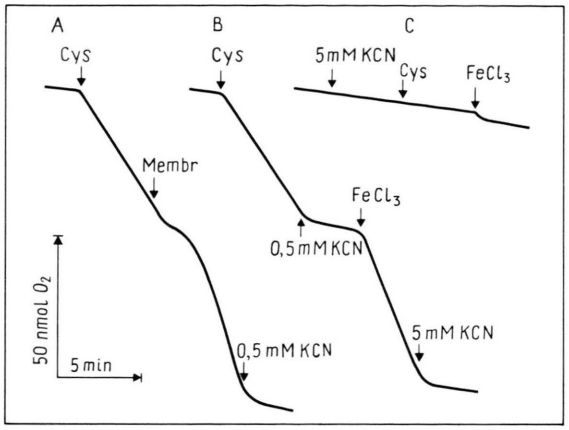

Fig. 1. Cysteine-induced O_2 uptake in 20 mM HEPES/NaOH buffer solution (pH 7.0). Additions: 5 mM cysteine, *A. nidulans* membranes with a chlorophyll content of 60 μg · ml^{-1}, 1 μM $FeCl_3$, KCN as indicated.

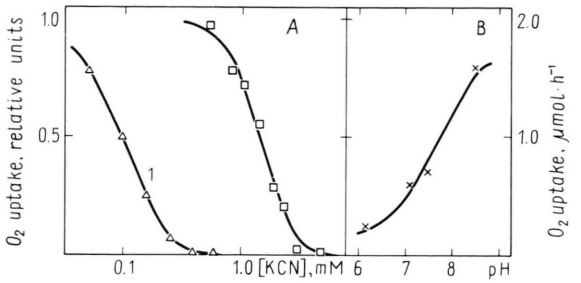

Fig. 2. Effect of CN^- (A) and pH (B) on the rate of cysteine-induced O_2 uptake. (A) 20 mM HEPES/NaOH buffer solution (pH 7.0) contained 5 mM cysteine. 1, without $FeCl_3$; 2, with 1 μM $FeCl_3$. The initial rate of O_2 uptake were equal to 0.55 and 0.9 μmol of $O_2 \cdot h^{-1} \cdot ml^{-1}$ in experiments 1 and 2 respectively. (B) The pH value of the solution containing 5 mM cysteine was varied by the addition of NaOH to 20 mM solutions of MES (pH 6.3) and HEPES (pH 7.1 and 7.5) and by the addition of MES to 20 mM solution of Tris (pH 8.5).

Table I. Effect of inhibitors on the O_2 uptake under oxidation of cysteine or ascorbate and TMPD. Incubation mixture: 20 mM HEPES/NaOH buffer (pH 7.0), 5 mM cysteine or 5 mM sodium ascorbate and 0.5 mM TMPD, *A. variabilis* membranes with a chlorophyll content of 65 μg · ml^{-1} in experiments with cysteine and 27 μg · ml^{-1} in experiments with ascorbate and TMPD. The rate of the O_2 uptake in the absence of inhibitors for cysteine or ascorbate + TMPD oxidation was equal to 0.48 and 0.21 without membranes and in the presence of the membranes to 1.12 and 1.24 μmol · h^{-1} · ml^{-1} respectively.

Additions	Concentration [mM]	Rate of the O_2 uptake (%) in the presence of			
		Cysteine		Ascorbate + TMPD	
		Without membranes	With membranes	Without membranes	With membranes
Azide	5.0	105	98	95	5
Cyanide	0.5	5	2	n.d.	n.d.
Cyanide	2.0	n.d.*	n.d.	105	35
Benzylhydroxamate	10.0	60	45	95	27
Cyanide + benzylhydroxamate	2.0 + 10.0	5	2.8	95	1.6
Hydroxylamine	5.0	42	40	100	1.0

* Not determined.

concentration of 0.5 mM completely inhibited the O_2 uptake. Similar results were obtained with *A. variabilis* membranes and have been reported by Schmidt and Krämer [6]. In our experiments CN^- inhibited O_2 uptake with cysteine independently of presence of the membranes (Fig. 1 A and B). 1 μM $FeCl_3$ abolished the inhibitory effect of CN^- on the O_2 uptake which was renewed by CN^- at a concentration of 5 mM (Fig. 1 B). CN^- prevents O_2 uptake in the presence of cysteine and $FeCl_3$ (Fig. 1 C).

As seen from Fig. 2 A, concentrations of CN^- necessary for half the inhibition of O_2 uptake in the presence of cysteine without (curve 1) and with $FeCl_3$ (curve 2) differ substantially and correspond to 0.1 and 1.5 mM respectively. The rate of autoxidation of cysteine increased with increasing pH of the solution (Fig. 2 B).

The data on the influence of Fe^{3+} and pH indicate that the oxidation of cysteine may be catalysed by the cations of heavy metals. CN^- is known to form

stable complexes with di- and trivalent cations of transition metals under reducing conditions [9]. In particular, cyanoferrates are formed in the presence of Fe^{3+}. The cyanide-bound ions of metals can not apparently further participate in oxidation of cysteine.

A comparative study of the effect of different inhibitors on oxidation of cysteine or ascorbate and TMPD was conducted in the final experiments (Table I). CN^-, benzylhydroxamate, their combination and NH_2OH (but not N_3^-) inhibited O_2 uptake with cysteine regardless of the presence of membranes to the same extent. None of the agents mentioned influenced the nonenzymic oxidation of ascorbate in combination with TMPD. However, respiration of membranes incubated with ascorbate and TMPD was effectively inhibited by N_3^-, CN^-, benzylhydroxamate and NH_2OH. This is in good agreement with the data of [3–5] and indicated that ascorbate and TMPD are oxidized *via* cytochrome aa_3 complex and alternative oxidase. The same inhibitory effect of CN^-, benzylhydroxamate and NH_2OH on the oxidation of cysteine in the presence and in the absence of membranes as well as the lack of inhibition by N_3^- points to a nonenzymic mechanism of oxidation of the cysteine added to the suspension of the membranes. Inasmuch as benzylhydroxamate and NH_2OH are complexones of some metals [9], they apparently prevent the catalytic effect of metals on oxidation of cysteine as well as CN^-.

Thus, ascorbate with TMPD is oxidized in the respiratory chain of cyanobacteria while cysteine is not a suitable (oxidizable) substrate for cytochrome oxidase. Increasing the rate of cysteine oxidation in the presence of isolated cyanobacterial membranes may be caused by accompanying cations of transitional metals.

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[1] G. A. Peschek, Biochim. Biophys. Acta **635,** 470–475 (1981).
[2] G. A. Peschek, Biochem. Biophys. Res. Commun. **98,** 77–79 (1981).
[3] P. F. Kienzl and G. A. Peschek, Plant Physiol. **69,** 580–584 (1982).
[4] G. A. Peschek, G. Schmetterer, G. Lauritsch, W. H. Nitschmann, P. F. Kienzl, and R. Muchl, Arch. Microbiol. **131,** 261–265 (1982).
[5] G. A. Peschek, Arch. Microbiol. **125,** 123–131 (1980).
[6] A. Schmidt and E. Krämer, Z. Naturforsch. **38 c,** 446–450 (1983).
[7] W. A. Kratz and J. Myers, Am. J. Bot. **42,** 282–287 (1955).
[8] J. P. Thornber, Biochim. Biophys. Acta **172,** 230–241 (1969).
[9] F. Umland, A. Janssen, D. Thierig, and G. Wünsch, Theorie und Praktische Anwendung von Komplexbildnern. Akademische Verlagsgesellschaft, Frankfurt/Main 1971.