FERREDOXIN-LINKED NITRATE REDUCTASE FROM THE PHOTOTROPHIC BACTERIUM *ECTOTHIORHODOSPIRA SHAPOSHNIKOVII*

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1. Introduction

Only a few species of phototrophic bacteria using nitrate as a source of nitrogen for biosynthesis are known [1–4]. Recently such a capacity has been found by us in purple sulphur bacterium *Ectothiorhodospira shaposhnikovii.*

This paper presents evidence that its nitrate reductase is dependent of ferredoxin.

2. Materials and methods

The cultures of *E. shaposhnikovii* strain N 1, grown in anaerobic conditions (atmosphere of H_2) in the light in Larsen's medium [5], containing 0.2% malate and 0.05% glutamate, 0.1% NH_4Cl or 0.1% KNO_3.

Cells were collected by centrifugation, washed with 0.1 M phosphate buffer (pH 7.8) and disrupted with a sonic disintegrator MSE (20kHz for 5 min). The nitrate reductase activity was assayed by measuring nitrite formation crude extract obtained by centrifugation of the sonicate at 4000 *g* for 1 hr. It was also assayed in the supernatant and particulate fraction received after centrifugation of crude extract at 14 400 *g* for 2 hr.

The reaction mixture contained 100 mol of KNO_3, 0.1–0.5 ml enzyme preparation (1–2 mg protein), 0.2–0.8 mM of electron donor per 2.5 ml. As electron donors NADH, HADPH, flavines ($FADH_2$ and $FMNH_2$), reduced animal cytochrome *c*, ferredoxin, sodium dithionite, methylene blue, phenazinemethasulphate and methyl viologen were used.

Dyes and flavines were reduced with freshly prepared 0.05% dithionite solution and cytochrome *c* with ascorbate in the presence of 2.4-dinitrophenolindophenol. The ferredoxin isolated from *E. shaposhnikovii* [6] was reduced with chloroplasts of pea leaves under argon atmosphere on light (1000 lx) at 25°C. Protein was determined by the method of Lowry et al. [7].

3. Results and discussion

The activity of nitrate reductase was considerable only in extracts of cells grown in the medium containing KNO_3. Greater activity was displayed by cells in the medium supplemented with 50–100 mg/l of sodium molibdate.

Of all electron donors used only methyl viologen and ferredoxin caused reduction of NO_3^- to NO_2^-. The addition of NAD and FAD together with reduced ferredoxin did not increase the nitrate formation (table 1).

The formation of NO_2^- by crude extracts in the presence of reduced methyl viologen took place in strict anaerobic conditions and in the presence of air and did not depend on illumination. The heating of extract at 60°C 5 min did not diminish its nitrate reducing potency. After ultracentrifugation of crude extract (144 000 *g*, 2 hr) up to 70% of nitrate reductase activity was found in particulate fraction containing chromatophores (table 2). But after washing with phosphate buffer this capacity of particulate fraction disappeared.

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Table 1
Nitrate reductase activity in crude extract of cells

The composition of reaction mixture	Amounts of NO_2^- formed μg/mg protein/hour
I. Complete system with reduced methyl viologen	0.387
−NO_3^-	0.030
−dithionite	0.0
−methyl viologen	0.031
−extract	0
II. Complete system with reduced ferredoxin	1.203
+NAD	1.125
+NAD + FAD	1.125
−extract	0.550
−chloroplasts	1.000
−ferredoxin	0.003

The composition of complete reaction mixture see (Materials and methods)

The evidence prestented above allows the following conclusions to be made. In contrast to nitrate reductase of nonsulfur purple bacterium *Rhodopseudomonas spheroides* showing activity in the presence of NADH [2], the nitrate reductase of *E. shaposhnikovii* is dependent on reduced ferredoxin as H-donor.

Until recently there was no information on ferredoxin dependent nitrate reductase. But recently there appeared a publication concerning existence of ferredoxin-linked nitrate reduction in an obligate anaerobe *Clostridium perfringens* [8].

According to all other observation ferredoxin participates in reduction of hydroxylamine in microorganisms and in reduction of nitrite in plants [9,10]. Thus, up to now *E. shaposhnikovii* seems to be the only phototrophic organism for which there are reasons to suppose the direct participation of ferredoxin in nitrate reduction.

The observations presented confirm as well the important role of ferredoxin in metabolism of phototrophic bacteria. Thus it seems possible to add one more reaction to the number of known ferredoxin dependent reactions in these microorganisms.

Table 2
Nitrate reductase activity in fractions of cells in the presence of reduced methyl viologen (μg NO_2^-/mg protein/hr)

Fractions	Experiments I	Experiments II
Crude extract (4000 *g*)	0.126	0.122
Supernatant (144 000 *g*)	0.886	0.155
Particulate fraction	2.300	0.406
Washed particulate fraction	–	0

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