ACTA BIOCHIMICA POLONICA
Vol. 39 1992 No.1

ZOFIA DZIERŻEWICZ, BEATA CWALINA and BOŻENA GAWLIK

EFFECT OF COPPER IONS ON HYDROGENASE ACTIVITY OF *DESULFOVIBRIO DESULFURICANS*

Department of Biochemistry and Biophysics, Silesian Medical Academy Narcyzów 1, 42-200 Sosnowiec, Poland

There are only limited data in the literature concerning the influence of transition metal ions upon metabolic processes in sulfate reducing bacteria (SRB) of *Desulfovibrio* and *Desulfotomaculum*. These bacteria are highly corrosive both towards metals and their alloys [1 - 4], and are involved in corrosion of underground pipelines and telecommunication or power cables [5]. Generally, biocorrosion is explained by usual cathode depolarization. It is assumed that SRB remove cathode hydrogen from iron surface thus making it depolarized and exposed to intense corrosion [6]. The depolarization of cathode capacity is thought to be proportional to hydrogenase activity [7]. It is known that two kinds of hydrogenase exists in SRB, and that they are localized in periplasmic and cytoplasmic spaces. It has been postulated that the hydrogen produced by the cytoplasmic hydrogenase diffuses across the cytoplasmic membrane and then is oxidized by periplasmic hydrogenase. Both kinds of these enzymes are necessary in intracellular hydrogen cycling for generation of a proton gradient in bacterial cells [8].

The specific activity of both periplasmic and cytoplasmic hydrogenases was measured during bacteria cultivation in a liquid or solid Postgate's medium [9, 10] free of SO_4^{2-} ions but enriched with 5×10^{-4} M $CuCl_2$ in liquid medium or 1×10^{-3} M $CuCl_2$ in the solid one. Control media were free of Cu^{2+}. The activity of cytoplasmic and periplasmic hydrogenase,

which were contained in respective leakages, as well as total activity of both hydrogenases in the crude extract of SRB, have been studied.

The crude cell extract was a supernatant obtained after ultrasonic disintegration of cells (20 000 Hz, 5 × 3 min, 0°C) followed by cell homogenate centrifugation (25 000 × *g*, 30 min, 0°C)

Periplasmic enzyme was a mixture extracted with a mixture of 50 mM Tris/HCl, pH 9, and 50 mM EDTA solution (10 cm^3/g of wet bacterial mass) [11]. The suspension was centrifuged for 30 min at 0°C (25 000 × *g*) and supernatant was used for specific activity determination. The bacterial residue obtained after centrifugation of the periplasmic hydrogenase was suspended in Tris/HCl buffer, pH 7.5 (2.5 cm^3/g of wet pellet mass), sonicated and centrifuged at 0°C for 30 min (25 000 × *g*). The supernatant was used for determination of specific activity of cytoplasmic hydrogenase. Hydrogenases were activated by bubbling hydrogen for 30 min at the flow rate of 4.5 cm^3/s. The activity was immediately determined spectrophotometrically with the use of methyl viologen and sodium dithionite [12]. Specific activity of enzyme was expressed as μmol H_2 evolved per minute per mg protein of crude cell extract, as well as of periplasmic or cytoplasmic leakages. Protein was determined by Lowry's method [13] with bovine serum albumin as standard.

As one can see from Table 1 Cu^{2+} added to the culture media suppressed bacterial growth and affected colour of the biomass.

Table 1

Effect of cupric ions on growth of Desulfovibrio desulfuricans DV-5/86

Concentration of Cu^{2+} 5×10^{-4} M and 1×10^{-3} M in the liquid and solid media, respectively

Features of cultures	Wet mass (g)	
	without Cu^{2+} (control)	with Cu^{2+}
From liquid culture	4.70	0.95
From solid culture	1.60	0.46
Appearance of culture on agar	layer of milky colonies	gold separated colonies

Table 2

Effect of cupric ions on hydrogenase in crude cell extract of Desulfovibrio desulfuricans DV-5/86 cultured on agar in the presence of Cu^{2+} (1×10^{-3} M)

Hydrogenase	Activity (μmol $H_2 \times min^{-1}$ per mg protein)	
	without Cu^{2+} (control)	with Cu^{2+}
Without activation	0.75	3.55
After activation by H_2	2.39	0.99

However, hydrogenase activity in the crude cell extracts was stimulated by Cu^{2+} (Table 2); in the control culture, the total hydrogenase was one-fifth that in the cultures cultivated in the presence of 1×10^{-4} M Cu^{2+}. On the other hand Cu^{2+} prevented activation of the enzyme by hydrogen.

The periplasmic and cytoplasmic enzymes showed rather similar specific activity but exhibited different response to Cu^{2+} activation; activation of the former was about 20-fold and that of the latter only 8-fold. The periplasmic enzyme also was activated by hydrogen (about 4-fold) but the presence of Cu^{2+} almost completely abolished this activation. On the other hand, the cytoplasmic hydrogenase was but slightly activated by hydrogen and in the presence of Cu^{2+} somewhat enhanced activation was observed.

The data available so far describe the effect of copper ions on the activity of the isolated and purified hydrogenases from SRB [12]. Cu^{2+} was found to abolish activation of periplasmic hydrogenase in *Desulfovibrio gigas* [14] by molecular hydrogen.

On the other hand, when a $CuCl_2$ solution saturated with hydrogen was added, the hydrogenase activity decreased even to the initial level [14] whereas no such effect was observed when Cu^{2+} ions were added immediately after isolation of the enzyme. A significant decrease in the enzyme activity occurred also on the addition of sodium ascorbate. In this case inactivation was ascribed to the action of cuprous ions [14].

The results presented in our paper are in agreement with the data obtained with the isolated enzymes *in vitro*. Both the periplasmic and cytoplasmic hydrogenases from the cells grown in the presence of Cu^{2+} showed higher activity than did the controls.

This effect of Cu^{2+} may be beneficial and counteracts inactivation since under aerobic conditions hydrogenase is insensitive to oxygen conditions. Such an active form of hydrogenase resistant to oxygen may be due to high affinity of Cu^{2+} to amide groups in the polypeptide chain as well as to imidazole of histidine and sulphydryl groups of cysteine residues [14]. Hydrogen in these ligands is easily substituted by copper ions with formation of stable 5- or 6-membered chelate rings. Formation of these form of enzyme could make impossible their activation by hydrogen in our experiments whereas the enzyme from our control culture was readily activated by hydrogen.

The bacteria grown in the presence of Cu^{2+} showed a significant decrease in hydrogenase activity following exposure to hydrogen. This can, probably, be accounted for by reduction of Cu^{2+} not only to Cu^{+} but also

Table 3

Effect of cupric ions on the specific activity of periplasmic and cytoplasmic hydrogenase in Desulfovibrio desulfuricans DV-5/86 cultured in the presence of 5×10^{-4} *M* Cu^{2+}

Hydrogenase		Specific activity (μmol $H_2 \times min^{-1}$ per mg protein)	
		without Cu^{2+} (control)	with Cu^{2+}
Periplasmic	non-activated	3.25	65.33
	activated by H_2	14.82	5.00
Cytoplasmic	non-activated	1.58	17.44
	activated by H_2	2.45	9.17

metallic copper as it appears quite clearly from the change in colour of the colonies grown on solid medium (Table 1).

REFERENCES

1. *Biologically induced corrosion* (1986) (Dexter, S. G., ed.), pp. 31 - 114. NACE, Houston, Texas.

2. Cwalina, B., Dzierżewicz, Z., Zawada, Z. & Wilczok, T. (1988) *Postępy Mikrobiologii*, **27**, 254 - 258.
3. Dzierżewicz, Z., Cwalina, B. & Zawada, Z. (1988) *Ochrona przed korozją*. **31**, 289 - 305.
4. Dzierżewicz, Z., Cwalina, B. & Zawada, Z. (1989) *Ochrona przed korozją*. **32**, 176 - 179.
5. Kozlova, I. A., Andreyuk, E. J., Antonovskaya, N. S., Razhanskaya, A. M. & Shevchenko, S. I. (1980) *Proc. Int. Conf. Use Microorg. Hydrometall., Hung. Acad. Sci. Local Comm. Pecs, Hung.*, 197 - 202.
6. Wolzogen Kühr von, G. A. M., Van der Vlugt, I. S. (1934) *Water* (den Haag), **18**, 147- 165.
7. Tiller, A. K. (1983) in, *Microbiological Corrosion;* pp. 54 - 65. The metals society, Delco Printing Company Limited, London.
8. Rohde, M., Fürstenau, U., Mayer, F., Przybyła, A., Peck, H., Le Gall, J., Choi, E. & Menon, N. (1990) *Eur. J. Biochem.*, **191**, 389 - 396.
9. Postage, J. R. (1984) *The sulphate-reducing bacteria;* pp. 24 - 40. Cambridge University Press.
10. Booth, G. H. (1971) *Microbiological Corrosion*; pp. 13 - 41. Mills & Boon Ltd., London.
11. Van der Vesten, H. D. & Mayhew, S. G. (1978) *FEBS Lett.*, **86** (1), 122 - 126.
12. Peck, H. D., Jr. & Gest, H. (1956) *J. Bacteriol.*, **71**, 70 - 80.
13. Kłyszejko-Stefanowicz, L. (1982) *Ćwiczenia z biochemii*; pp. 245 - 246. PWN, Warszawa - Poznań.
14. Fernandez, V. M., Rua, M., Reyes, P., Cammack, R. & Hatchikian, E. G. (1989) *Eur. J. Biochem.*, **185**, 449 - 454.