FORMATE : A NEW ELECTRON DONOR FOR NITRITE REDUCTION IN ESCHERICHIA COLI K12

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SUMMARY

Reduction of nitrite by unbroken cells of Escherichia coli occurs with formate as electron donor. This activity constitutes about 25 % of the overall nitrite reduction. Electron transfer is performed through the formate dehydrogenase and, probably, a cytochrome-containing chain.

INTRODUCTION

Reduction of nitrite by unbroken cells of Escherichia coli is readily achieved using glucose as donor. This provides a good estimation of the overall nitrite reductase activity but does not give any indication on the pathways involved in the electron transport or on the nature of the electron donors. In cell-free extracts, two nitrite reductases have been described (1). The first is an NADPH-sulfite reductase (EC 1.8.1.2.) using nitrite as substrate. The second is an NADH-specific enzyme (EC 1.6.6.4) which has been purified recently (2). Assays of nitrate-reduction by unbroken bacteria using formate as donor lead us to find out that this compound is actually a good electron donor for nitrite reduction. In this paper, we report on some preliminary results on the properties of formate-nitrite reductase system. Subsequent communications will describe the physiological properties of this system and its relations with formate-nitrate reductase and hydrogenlyase systems.

Abbreviation used : 2-Heptyl-4-Hydroxyquinoline N-oxyde, HQNO.

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Table 1. List of strains

Number	Polarity	Genotype
CB10	F^-	thr_1, leu_6, B_1, lac Y1, sup E44, ton A21, ana_1, fdh A, str.
CB303	Hfr P4X	met B_1
CB322	Hfr H	B_1
CB356	F^-	B_1, thr_1, leu_6, his, arg BCEH, purE, pro, lac Y_1, mal A_1, xyl_7, ara_{13}, mtl_2, gal_6, ton A_2, $/T_1$, str.
CB567	F^-	B_1, ilv_7, his_4, aro A, arg_3, pro_2, gal, xyl, str.
CB900	F^-	thr_1, leu_6, B_1, lac Y1, sup E44, ton A21, ana_1, str.

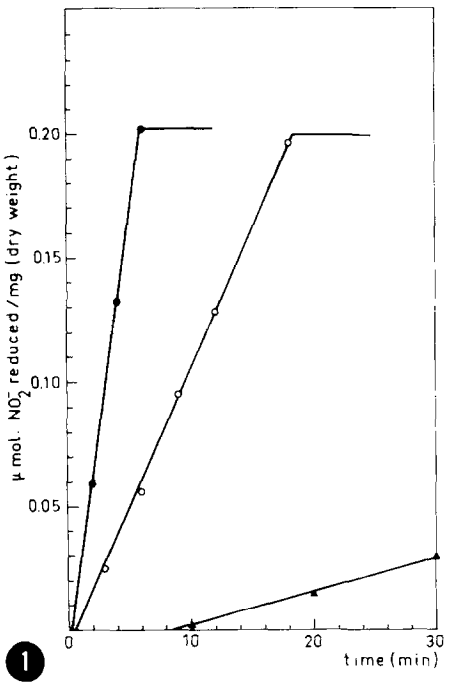
MATERIALS AND METHODS

Strains used in this work are listed in Table 1. Medium used for bacterial cultivation contains per liter distilled water : KH_2PO_4, 0.98 g ; Na_2HPO_4, 12 H_2O, 3.358 g ; NH_4Cl, 0.5 g ; trace salts ; peptone, 0.5 g ; yeast extract, 0.5 g ; glucose 2 g and KNO_2, 0.085 g.

Bacteria were grown anaerobically at 37° C and harvested before all nitrite was exhausted. Cells were washed twice with and resuspended in 15 mM phosphate (pH 7). Rates of nitrite reduction by suspensions of bacteria were assayed in open test tubes containing : 15 mM phosphate (pH 7) ; 0.5 mM KNO_2 ; approximately 9.3 mg dry weight of bacteria ; 40 mM glucose or formate. Final volume was 5 ml. At intervals, samples were tested for nitrite using the method described by Snell and Snell (4). For inhibition experiments HQNO was dissolved in dimethyl sulfoxyde.

RESULTS AND DISCUSSION

Rates of nitrite reduction with glucose and with formate as donors are shown on Fig. 1. It can be observed that, in both cases, nitrite is reduced at linear rate. The rate of reduction with formate as donor constitutes a significant part (20-30 %) of the overall nitrite reductase activity. The activity is a linear function of the amount of bacteria. On Fig. 2 are reported the rates of nitrite reduction with formate in the presence of inhibitors. It is clearly demonstrated that cyanide is a strong inhibitor whereas azide is not. The utilisation of formate as donor indicates that



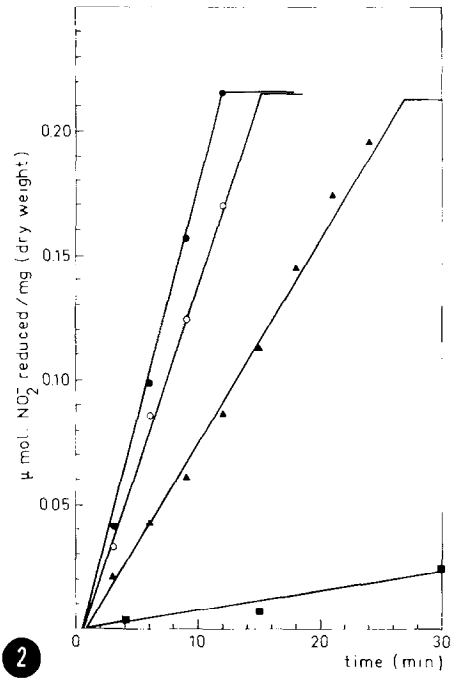


Figure 1 - Reduction of nitrite by unbroken cells of strain CB303
- ● with glucose as donor
- o with formate as donor
- ▲ without exogenous donor

Figure 2 - Effect of inhibitors on the formate nitrite reductase activity
- ● without inhibitor
- o with N_3^- at a final concentration of 10^{-3} M
- ▲ with HQNO at a final concentration of 10^{-4} M
- ■ with CN^- at a final concentration of 10^{-3} M

the formate dehydrogenase enzyme (EC 1.2.99.-) is involved in the electron transfer from formate to nitrite. This is confirmed by the following observation : strain CB900 possesses the formate-nitrite reductase activity. An fdh mutant of this strain (CB 10) lacks the formate dehydrogenase activity (Casse, F., unpublished data) ; it is found to be no longer able to reduce nitrite with formate (Table 2). Furthermore, the activity is inhibited by HQNO, which is known to be an inhibitor of cytochrome-containing respiratory chains. One can thus suppose that the electron transfer between formate

Table 2. Reduction of nitrite by unbroken cells of various strains of Escherichia coli K12

Strains	Electron donor	
	Formate	Glucose
CB303	0.7	2.2
CB322	0	1.7
CB356	0	1.3
CB567	0.4	1.8
CB900	0.53	2.2
CB10	0	1.9

Activities are expressed in μmoles/h/mg (dw)

and nitrite requires a complex transport system which could be in part common with the formate-nitrate electron transport system. Several strains of our collection have been tested for the formate-nitrite reductase activity ; Table 2 gives the data obtained with some of them. One can observe that most do possess the activity which then appears to be a common feature among E. coli K12 strains. Nevertheless, we have to point out that this activity is completely lost when the cells are disrupted.

In 1967, Fujita and Sato (5) reported the production of gas by nitrite-grown cells in response to nitrite addition. The gas was identified as CO_2 but the substrate from which it was derived remained unknown. The existence of the formate-nitrite reductase activity makes formate likely to be the source of the CO_2. Until now, only Cole and Ward (6) have mentioned the isolation of mutants affected in nitrite reduction but all the mutants reported to date have retained part of the activity measured with glucose. It would be interesting to know whether formate-nitrite reductase could not account for the residual activity.

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