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Effect of Electron Donors and Acceptors on Alcohol Dehydrogenase Activity during the Growth of *Escherichia coli*

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Alcohol dehydrogenase was activated by electron donors and inactivated by electron acceptors during the growth of *Escherichia coli*. A correlation was found between the k value, which expresses how much energy will be needed to remove an electron from a molecule, and the activity of this enzyme. The smaller the k value, the better the molecule in question acts as an electron donor, and the higher the alcohol dehydrogenase activity rises.

The electron donors obviously protect the enzyme molecule from the electron-catching effect of the environment. On the contrary, the electron acceptors catch electrons from the enzyme molecule, causing its inactivation.

At the turn of the 1930's and 1940's many workers were interested in the effect of the redox state on enzyme activity. They noticed that a number of different enzymes are activated by certain reducing agents (*e.g.* KCN, $Na_2S_2O_4$, H_2S, cysteine) and inactivated by oxidizing agents (*e.g.* H_2O_2, $KMnO_4$, $K_3Fe(CN)_6$). Such enzymes include amylase,[1] esterase and lipase,[2] ribonuclease,[3] and urease.[4] By contrast pepsin and pepsinogen are inactivated by reductants and reactivated by oxidants.[5] The hydrolytic activity of urease,[6] pancreatic lipase,[7] and liver esterase [8] is inactivated by oxidants and activated by reductants but their synthetic activity is affected by these agents in the opposite way. Sizer and Tytell [9] noticed that crystalline urease has a bell-shaped activity curve as a function of redox potential.

The workers referred above assumed that the effect of redox compounds on enzyme activity is due to oxidation and reduction of the sulfhydryl groups in the enzyme molecule and they studied this effect on enzyme activity *in vitro*.

In this work we have studied the effect of electron donors and acceptors on alcohol dehydrogenase activity *in vivo* during the growth of *Escherichia coli* by adding different agents to the growth medium.

MATERIALS AND METHODS

Organism. Escherichia coli U5-41, a wild type strain, details of the source and maintenance of which have previously been published.[10] The organism was precultured in one liter of medium in a 2 l Erlenmeyer flask at 37° without aeration. The composition of the medium was as described.[11] Culture was carried out at 37° in a shaker (Buhler Sm 1) in 1 l Erlenmeyer flasks containing 500 ml of minimum medium of the following composition: 0.1 % NH_4Cl, 0.7 % Na_2HPO_4, 0.3 % KH_2PO_4, 0.5 % NaCl, 0.01 % $MgSO_4.7H_2O$, 0.2 % glucose.

Redox effect experiments. Different amounts of different redox compounds were added to the test culture, usually at the early logarithmic phase. Growth was measured using a Klett-Summerson colorimeter with filter No. 62 (590 – 640 nm). Samples of 5 – 20 ml were taken from the test culture and reference culture, which did not contain any redox compound, at various time intervals for estimations of the enzyme activity. The cells of the samples were centrifuged at 4000 *g* for 10 min, washed twice with cold saline, and frozen at −25°.

Enzyme activity estimation. The cells were disintegrated for 3 min with a sonic oscillator (MSE Ultrasonic Disintegrator 60W, 20 kHz). The cell debris was centrifuged as above and the supernatant was used as enzyme preparation. The alcohol dehydrogenase activity was measured by the method of Vallee and Hoch.[12] Blank cuvettes contained all the reaction mixture components except ethanol. The protein determination for the specific activity estimations were carried out by the sulfosalicylic acid method of Heepe, Karte and Lambrecht, as modified by Heinonen.[13]

RESULTS AND DISCUSSION

Effect of a strong reductant or oxidant on the alcohol dehydrogenase activity during growth. After addition of sodium dithionite, $Na_2S_2O_4$, in a concentration of 5 mM to the growth medium of *E. coli* at the beginning of the logarithmic phase, the specific activity, which normally decreases at the end of the logarithmic phase, increased and remained at a high level. The growth of *E. coli* was slower in a dithionite-containing culture than in the normal medium.

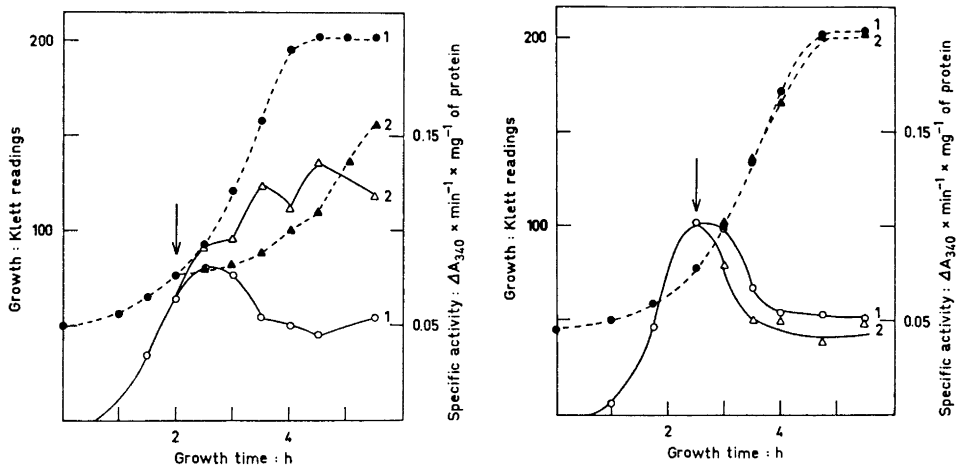

Fig. 1. Addition of $Na_2S_2O_4$ (A) and KNO_3 (B) to the concentration of 5 mM (dithionite) and 50 mM (KNO_3) to the growth medium of *E. coli* at the time indicated by the arrow. Solid symbols = growth curves; open symbols = specific activity of alcohol dehydrogenase. 1 = no addition; 2 = addition of dithionite or KNO_3 at the time indicated by the arrow.

On the contrary, when an oxidant, KNO_3, was added in a concentration of 50 mM to the growth medium, the decrease in specific activity was accelerated. There was no difference in the growth curves (Fig. 1).

Effect of different electron donors and acceptors in different concentrations on alcohol dehydrogenase activity during growth. A number of different electron donors and acceptors were also tested. They were added in various concentrations to the growth medium at the beginning of the logarithmic phase and samples were taken at the end of the logarithmic phase. The specific alcohol dehydrogenase activity of the test samples was estimated and the increase or decrease in relation to the normal specific activity was calculated and is presented as percentages in Table 1.

Table 1. Effect of different electron donors and acceptors in different concentrations on alcohol dehydrogenase activity during the growth of *E. coli*. The percentages are deviations from the initial activity which was taken as 100.

Compound	Concentration mM	Activity %	Compound	Concentration mM	Activity %
1. $KMnO_4$	0.5	−100	8. Catechol	50	−100
»	0.1	−18	»	10	20
»	0.01	31	»	1	37
2. KIO_4	25	−100	9. L-Histidine	50	13
»	10	−88	»	10	28
»	1	−29	»	1	35
3. KNO_3	50	−26	10. Cytosine	50	15
»	10	−34	»	10	64
»	1	−26	»	1	64
4. Oxidized glutathione	10	−8	11. L-Tryptophan	25	50
»	1	−8	»	10	53
»	0.1	−31	»	1	85
5. $Na_2S_2O_4$	10	46	12. Ascorbic acid	50	15
»	1	43	»	10	28
»	0.1	28	»	1	30
6. Na_2S	10	21	13. Thymine	30	93
»	1	41	»	10	93
»	0.1	14	»	1	100
7. L-Phenylalanine	50	1	14. *p*-Phenylenediamine	50	95
»	10	3	»	10	41
»	1	16	»	1	23

It is seen from Table 1 that both inorganic and biochemical electron donors in different concentrations activated alcohol dehydrogenase. Similarly, the electron acceptors inactivated the enzyme. It should be noted that the results are from separate cultures. The following experiment was made in one culture and the values are comparable to each other.

Correlation between the k value and the increase in alcohol dehydrogenase activity during growth. Szent-Györgyi [14] has listed the *k* values calculated by

A. and B. Pullman and G. Karreman for a number of molecules taking part in different biological reactions. The k value for the highest filled orbital indicates how much energy will be needed to remove an electron. The smaller its value, the more readily the molecule in question will give up this electron and act as an electron donor. We have tried to determine how the k values correlate with the increase in alcohol dehydrogenase activity during the growth of *E. coli*. The following substances (k values in brackets) were tested: *p*-hydroquinone (1.000), L-phenylalanine (0.908), L-histidine (0.660), cytosine (0.595), L-tryptophan (0.534), thymine (0.510), and *p*-phenylenediamine (0.321). Each compound was added in a concentration of 30 mM in the beginning of the logarithmic growth phase of *E. coli* and samples were taken at the end of the logarithmic phase. In Fig. 2 we can see that these is a correlation between the k value and the enzyme activity in the cells. Low k values gave the highest activities.

Addition of an electron donor at different points of the growth cycle of E. coli. We added L-tryptophan, which decreases the inactivation of the specific activity of alcohol dehydrogenase during the growth cycle, to the concentration of 30 mM at three different points during the growth cycle, *viz.* at the acceleration phase, in the beginning of the logarithmic phase and in the middle of the logarithmic phase, to the growth medium of *E. coli*. The growth of the organism and the variations in the specific alcohol dehydrogenase activity

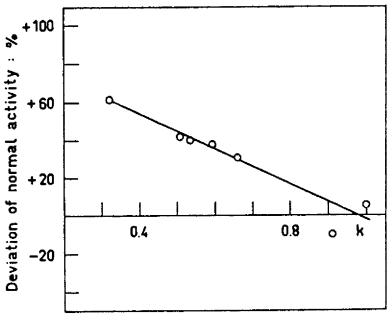

Fig. 2. Correlation between the k value and the specific alcohol dehydrogenase activity during the growth of *E. coli*. Experimental details, see Results and Discussion. Deviations from normal activity are expressed as percentages (see Table 1).

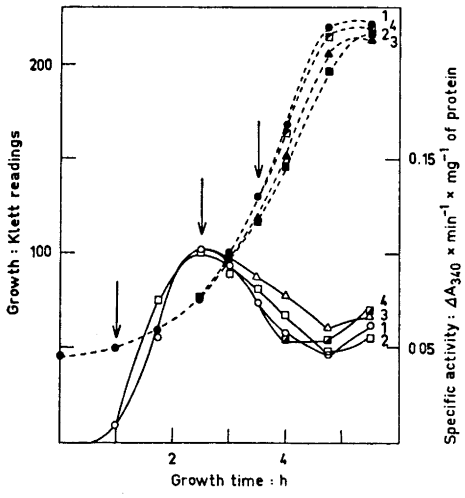

Fig. 3. Addition of L-tryptophan to the concentration of 30 mM to the growth medium of *E. coli* at three different times indicated by the arrows. Solid symbols = growth curves; open symbols = specific activity of alcohol dehydrogenase. 1 = no addition; 2 = addition at the growth time of 1 h; 3 = addition at the growth time of 2.5 h; 4 = addition at the growth time of 3.5 h.

are presented in Fig. 3. We can see that if an electron donor is added at the acceleration phase the enzyme activity will rise slightly faster and remain higher than in the normal medium. Similarly, if the addition is made in the beginning of the logarithmic phase, when the enzyme activity is highest, the fall of the activity will clearly be slower. On the other hand, when tryptophan is added in the middle of the logarithmic phase, when the enzyme activity has fallen to half the total decrease, there is not such a clear retarding effect. Only in the stationary growth phase is the activity higher than in the reference culture. We do not get total protection of alcohol dehydrogenase, as with dithionite, but on the other hand the k value of tryptophan is higher.

The explanation offered here to account for these results is that normally at the end of growth electrons are removed from the enzyme protein causing enzyme inactivation. When we add an electron donor to the medium, it donates electrons, thus leaving the enzyme protein intact. On the contrary, when we add an electron acceptor to the medium, enzyme inactivation will be faster because the acceptor will take the electrons from the enzyme protein, too.

The most usual reason for inactivation of an enzyme by oxidants and reactivation by reductants is the presence of thiol groups in the enzyme molecule. When the sulfur is in the reduced $-SH$ form the enzyme is active, whereas in the oxidized $-SS-$ form the enzyme is inactive. We assume that these are other labile groups in the enzyme molecule, too. In this work we have seen that certain free amino acids, tryptophan, histidine and phenylalanine, are rather good enzyme protectors as well as being good electron donors. It is possible that these amino acids in the enzyme molecule will also donate electrons. That might cause alteration in the enzyme protein conformation which changes the active site of the enzyme causing the enzyme inactivation. One also has to pay attention to the regulatory effect on enzyme activities of several biologically important electron donating compounds, such as the amino acids mentioned in this paper, purines and pyrimidines.

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