PHYSIOLOGICAL FUNCTIONS OF NAD- AND NADP-LINKED MALIC ENZYMES IN ESCHERICHIA COLI

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SUMMARY: Regulatory mechanisms for biosyntheses of NAD- and NADP-linked malic enzymes in E. coli cells were studied. The NAD-enzyme was repressed by glucose and induced by malate. The repression by glucose was overcome by the addition of malate. In contrast, the NADP-enzyme was repressed by glucose, glycerol, lactate or acetate in decreasing order of magnitude, in the absence and presence of malate which gave a high level of the enzyme. These results, together with ^{14}C-incorporation experiments into fatty acids from 1,4- or 2,3-^{14}C-labeled succinate by using resting cells and the results so far reported, suggest that the NAD-enzyme takes a role in the catabolism of malate, while the NADP-enzyme, in the supply of acetyl-CoA from malate via pyruvate.

Up to the present time, there have been reported some examples of two separate dehydrogenases requiring NAD and NADP in one organism. One of the typical examples is a pair of glutamate dehydrogenases from yeast (1), Neurospora (2) and Thiobacillus (3). Concerning their physiological roles in cell, it is established that NAD-linked glutamate dehydrogenase takes a part in the catabolism of glutamate (1,3), while the NADP-linked enzyme in the biosynthesis of glutamate (4).

In 1967 the presence of NAD- and NADP-linked malic enzymes in E. coli W was reported from our laboratory (5). As the physiological role of malic enzyme, the following various functions have been speculated: fixation of carbon dioxide to form malate (6); degradation of malate to form acetyl-CoA via pyruvate (7,8,9);

NADPH production for lipogenesis and other biosynthetic reactions (10,11). Nevertheless, no study has been made on the physiological functions of such a pair of malic enzymes in relation to each other.

Brice and Kornberg (12) found that a mutant of E. coli lacking phosphoenolpyruvate carboxylase could not grow on a glucose medium unless any of the organic acids in the tricarboxylic acid cycle was added to it. This observation indicates that malic enzyme does not operate in the CO_2-fixation reaction in E. coli under the usual conditions. As another important information, it was found by our group (13) and Sanwal et al. (14) that the NAD- and NADP-enzymes are both allosteric enzymes, being controlled by aspartate as activator and by acetyl-CoA as inhibitor, respectively.

This communication deals with the controlling factors of the biosyntheses of the two malic enzymes in E. coli in relation to each other and presents some observations concerning the metabolic flow of malate in addition. Presumption is made on the physiological roles of the two malic enzymes based on the results obtained.

RESULTS AND DISCUSSION

E. coli W was grown on media containing various compounds as carbon source and harvested at late logarithmic growth phase. Cell-free extracts were prepared by sonic disruption of the cells and centrifugation at 105,000 x g for 120 min at 4°. The activities of malic enzymes were assayed spectrophotometrically with the cell-free extracts at 30° and one unit of the activity was defined as the amount producing one OD unit per min (5). The enzyme level was expressed in terms of the enzyme activity per mg of protein. The activity of NADH oxidase in the extracts was negligible.

Fig. 1 shows the levels of the two malic enzymes in the

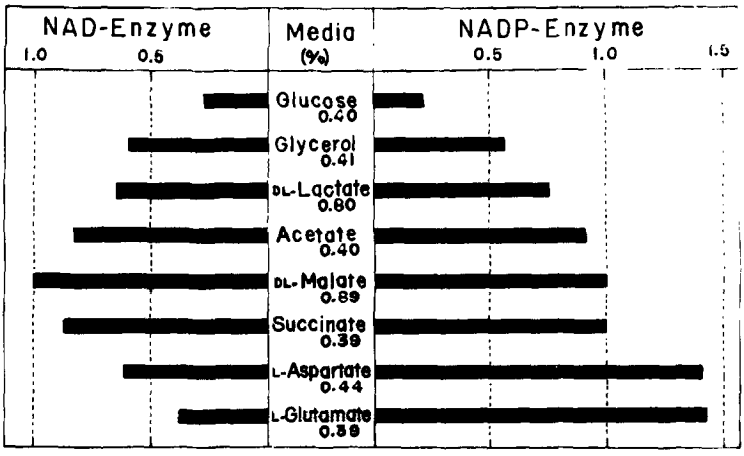

Fig. 1. Effect of various carbon sources on the levels of the two malic enzyme.
The reaction mixture for the assay of the NAD-enzyme contained the following constituents (μmoles in 3 ml): potassium L-malate, 20; $MnCl_2$, 3; NAD, 1; Tris-HCl (pH 7.8), 100; and the enzyme; and that of the NADP-enzyme contained the same constituents except for the use of 0.4 μmole of NADP and 100 μmoles of Tris-HCl (pH 9.0) as coenzyme and buffer, respectively. The enzyme level is indicated as relative value of enzyme activity per mg of protein.

extracts prepared from the cells grown on media containing various organic compounds as a sole carbon source. The lowest level was found in the cells from the glucose medium with both enzymes. The highest was found in the cells from the malate medium with the NAD-enzyme and in those from the aspartate- or glutamate medium with the NADP-enzyme. Repression of the enzymes could not be found in the acetate medium unlike the case of Pseudomonas (9), Rhodotorula (10), and Neurospora (11). From these facts, a question was raised whether the two enzymes are induced by malate or repressed by glucose. To solve this problem, the addition experiments of malate to the glucose medium were carried out. As shown in Fig. 2, a lower level was observed on the combined medium (glucose, 0.40% and malate, 0.89%) compared to that on the malate medium (malate, 0.89%) with both enzymes. However, the level of the NAD-enzyme was increased by increasing the concen-

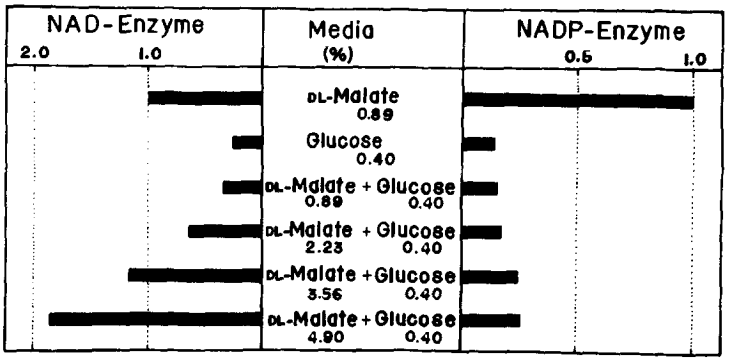

Fig. 2. Effect of addition of varying concentrations of malate to glucose medium on the levels of the two malic enzymes. The enzyme levels were determined as described in Fig. 1.

tration of malate without changing the concentration of glucose while no increment was observed with that of the NADP-enzyme. These results suggest the repression of both enzymes by glucose and the induction of the NAD-enzyme by malate.

Fig. 3 shows the effects of addition of glycerol, lactate or acetate, the intermediate compound in glycolysis or the related compound, to the malate medium on the levels of both enzymes. As can be seen from the figure, the level of the NADP-enzyme decreased to less than 60%, whereas that of the NAD-enzyme showed no appreciable change. It is a remarkable fact that the NADP-

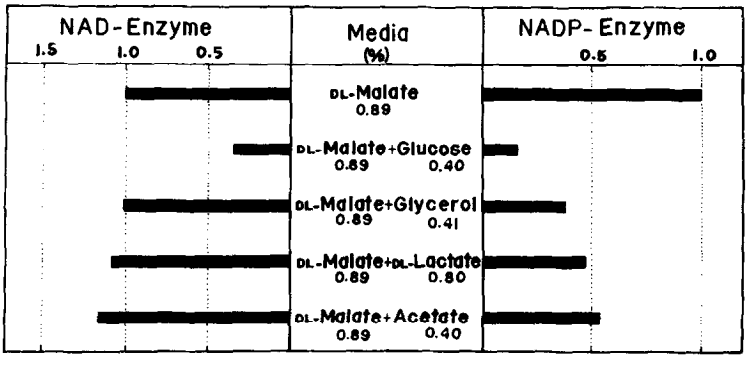

Fig. 3. Effect of addition of glycolytic intermediates to malate medium on the levels of the two malic enzymes. The enzyme levels were determined as described in Fig. 1.

enzyme showed a much lower level on the malate-acetate medium than that on the malate medium. From these results, the co-repressor of the NADP-enzyme is presumed to be acetyl-CoA because these three compounds (glycerol, lactate and acetate) are known to give acetyl-CoA and the reactions to yield acetyl-CoA from the former two compounds *via* pyruvate are essentially irreversible.

These results suggest that the NAD-enzyme undergoes an induction by malate, whereas the NADP-enzyme undergoes a repression by acetyl-CoA (nutritionally by acetate) in the presence of malate, and also indicate that both enzymes are repressed by glucose. Possible regulatory mechanisms of these two malic enzymes in *E. coli* are schematically shown in Fig. 4 based on the present results with the allosteric characters of the two enzymes (13,14). It is presumed that the NAD-enzyme takes a part in the catabolism of malate, controlling the levels of C_4-dicarboxy organic- and amino acids in the cell, whereas the NADP-enzyme, in the supply of acetyl-CoA

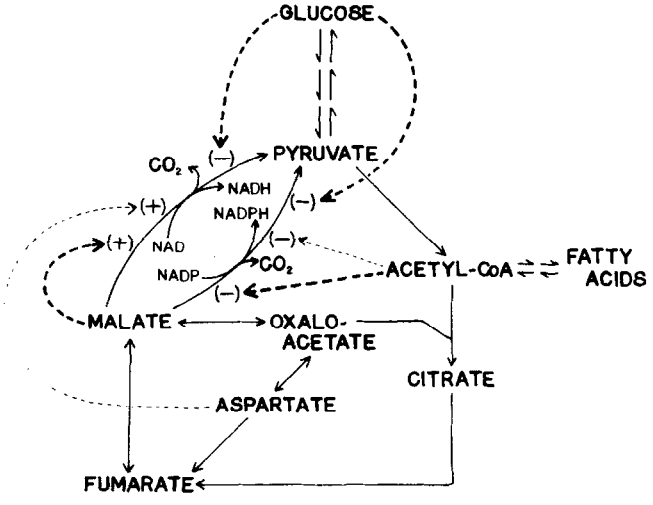

Fig. 4. Proposed regulatory mechanisms of NAD- and NADP-linked malic enzymes in *E. coli*.
The thick dotted lines indicate the controls of the synthesis of the enzymes, and the thin dotted lines, the allosteric controls of the enzyme activities.

from malate, which is utilized for the biosynthesis of lipid and other compounds as well as for the maintenance of the action of the tricarboxylic acid cycle. The NADPH which is produced through the reaction by the NADP-enzyme would be useful for anabolic metabolism of the cell at the same time.

As another enzyme involved in the reaction to form acetyl-CoA from malate, malate cleavage enzyme was reported from E. coli by Stern et al. (15). In order to investigate the possibility of the participation of the cleavage enzyme in the reaction, the

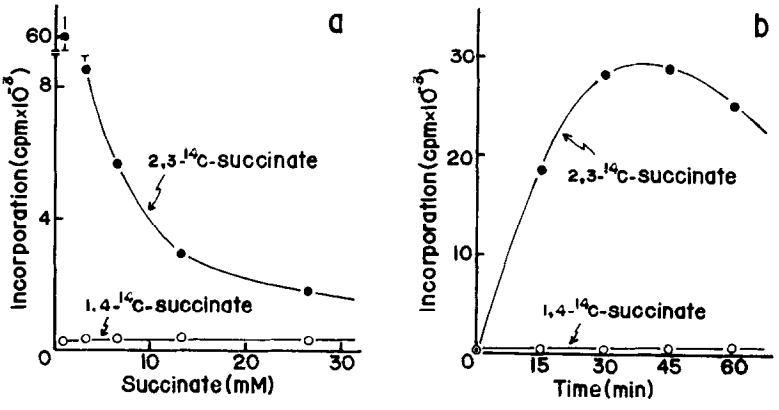

Fig. 5. Incorporation of radioactivity from 1,4-^{14}C- or 2,3-^{14}C-succinate into fatty acids. a)Effects of varying concentrations of succinate on the incorporation (incubation, 30 min; radioactivity, 566,000 cpm). b) Effect of incubation time on the incorporation (succinate, 1.0 μmole, 68,000 cpm). The cells grown on the minimum malate medium were harvested at logarithmic growth phase (OD_{660}=1.5) and washed twice with 0.1 M KCl. The optical density of the cell suspension in the minimum medium (5) from which carbon source was omitted was adjusted to 5.0 at 660 mμ. One ml of the suspension was preincubated with shaking at 30° for 30 min. To the suspension was added labeled and unlabeled succinate as indicated. 1,4-^{14}C-succinate and 2,3-^{14}C-succinate used were products of Daiichi Pure Chemicals Co., Ltd. (10.0 and 16.7 mCi per mmole, respectively). After dilution with water to 1.5 ml, the mixture was incubated with shaking at 30° for the indicated period. The incubation was stopped by the addition of 1.5 ml each of 50% KOH and methanol and the mixture was saponified for 3 hr at 70°. Acidic lipid was then extracted with ether by the usual method. The ethereal solution of acidic lipid was concentrated near to dryness. It was dissolved in 10 ml of scintillator (4 g of DPO, 0.1 g of POPOP and 120 g of naphthalene in 1 liter of 1,4-dioxane) and was counted for its radioactivity in Nuclear Chicago liquid scintillation spectrometer.

incorporation of radioactivity from 2,3-^{14}C- or 1,4-^{14}C-succinate into fatty acids was studied with the intact cells grown on the malate medium. If the incorporation from 1,4-^{14}C-succinate is demonstrated, the contribution of the cleavage enzyme to the formation of acetyl-CoA would be substantiated. On the contrary, if the degree of contribution of the malic enzyme(s) is higher than that of the cleavage enzyme, a higher incorporation should be seen from 2,3-^{14}C-succinate than 1,4-^{14}C-succinate. The results are shown in Fig. 5 (a and b). Evidently a much higher degree of incorporation was observed from 2,3-^{14}C-succinate than from 1,4-^{14}C-succinate, indicating that almost all the activities of acetyl-CoA formation from malate were ascribed to malic enzymes, but not to malate cleavage enzyme under the experimental conditions. This result indirectly supports the physiological roles of malic enzymes proposed.

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