Role of Phosphoenolpyruvate in the NADP-Isocitrate Dehydrogenase and Isocitrate Lyase Reaction in Escherichia coli

Tadashi Ogawa, Keiko Murakami, Hirotada Mori, Nobuyoshi Ishii, Masaru Tomita, and Masataka Yoshin

Department of Biochemistry, Aichi Medical University School of Medicine, Nagakute, Aichi 480-1195, Institute for Advanced Biosciences, Keio University, Tsuruoka, Yamagata 997-0035, and Nara Institute of Science and Technology, Ikoma, Nara 630-0101, Japan

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Phosphoenolpyruvate (PEP) is a critical metabolite in Escherichia coli, because it is essential for the efficient uptake of glucose and other carbohydrates (11) and the final intermediate of glycolysis. PEP acts as an effector of phosphofructokinase (1), and changes in the PEP concentration are related to the flux of the tricarboxylic acid (TCA) cycle and glyoxylate shunt (10, 13). The branch point at the TCA cycle and glyoxylate bypass is controlled by enzyme expression and depends on the growth conditions (16) and the NADP-isocitrate dehydrogenase activity (12), which is subject to inhibition by various metal ions (7, 14, 15) and phosphorylation/dephosphorylation of the protein (2, 9, 12). Here we report the inhibitory effects of PEP on the NADP-isocitrate dehydrogenase and isocitrate lyase purified from the archived clones of the icd- and aceA-overexpressing E. coli mutants (8), which were constructed with plasmid pCA24N from the E. coli strain K-12 W3110 (4).

The effect of PEP on the activity of NADP-isocitrate dehydrogenase was analyzed. The 1-ml reaction mixture contained 100 mM morpholinepropanesulfonic acid (MOPS) buffer (pH 7.1), 0.5 mM NADP, 0.5 mM MgCl2, and various concentrations of threo-D-S-isocitrate in the absence and presence of PEP (Fig. 1A). The substrate concentration required for half-maximal velocity, \( S_{0.5} \), increased to a value over 0.1 mM, and the Hill’s interaction coefficient, \( n_H \), remained constant at 1.1.

**FIG. 1.** Effect of phosphoenolpyruvate on the activity of E. coli NADP-isocitrate dehydrogenase with respect to the threo-D-S-isocitrate concentration. (A) threo-D-S-Isocitrate saturation curves in the absence and presence of different concentrations of phosphoenolpyruvate. (B) Hill’s plot of the data shown in panel A. Points represent experimental data, and lines are theoretically drawn by using the following equation: \( v = V \cdot \frac{[S]^n}{([S]^n + K_m^n)} \) where \([S]\) is the concentration of isocitrate, \( K_m \) is the concentration required for half-maximal velocity, and \( n \) is the Hill’s interaction coefficient. Symbols: closed circles, no addition (\( K_m = 0.029 \) mM and \( n_H = 1.0 \)); triangles, 0.2 mM PEP added (\( K_m = 0.046 \) mM and \( n_H = 1.1 \)); squares, 1 mM PEP added (\( K_m = 0.08 \) mM and \( n_H = 1.1 \)); open circles, 5 mM PEP added (\( K_m = 0.16 \) mM and \( n_H = 1.2 \)).

* Corresponding author. Mailing address: Department of Biochemistry, Aichi Medical University School of Medicine, Aichi 489-1195, Japan. Phone: 81-52-264-4811. Fax: 81-561-61-4056. E-mail: yoshino@aichi-med-u.ac.jp.

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FIG. 2. Inhibition of E. coli NADP-isocitrate dehydrogenase by phosphoenolpyruvate. Reaction conditions were similar to those in the legend to Fig. 1, except the following modifications. The PEP concentrations were varied, and the threo-β-S-isocitrate concentrations were fixed at 0.1 mM (circles), 0.02 mM (squares), and 0.004 mM (triangles). (A) Inhibition by phosphoenolpyruvate. (B) Variation of the quotient function \( Q \) with respect to the inhibition by PEP, assuming two binding sites for this inhibitor. The values of \( v^* \) taken in constructing this plot were determined from the velocity without PEP.

FIG. 3. Effect of phosphoenolpyruvate on the activity of E. coli isocitrate lyase with respect to the isocitrate concentration. (A) threo-β-S-Isocitrate saturation curves in the presence of different concentrations of phosphoenolpyruvate. (B) Hofstee plot of the data in panel A. The Hofstee equation for uncompetitive inhibition is as follows: 

\[
\frac{S}{V} = \frac{(1 + [I]/K_i)}{([S] + K_m/V_{max})} = \frac{1}{[I]/K_r} \cdot \frac{[S]}{V_{max}}
\]

The relationship between \( \frac{S}{V} \) and \( [S] \), the substrate concentrations in the presence of various concentrations of PEP, the inhibitor, \( [I] \) gave straight lines converging on the ordinate at \( K_m/V_{max} \). Points represent experimental data, and lines are theoretically drawn as demonstrated in Fig. 1 except that the \( n_v \) value was kept at 1. Symbols: open circles, no PEP (\( K_m = 0.89 \) mM and \( V_{max} = 6.72 \mu\text{mol/min per mg protein} \)); closed circles, 0.2 mM PEP added (\( K_m = 0.72 \) mM and \( V_{max} = 4.92 \mu\text{mol/min per mg} \)); triangles, 1 mM PEP added (\( K_m = 0.47 \) mM and \( V_{max} = 3.29 \mu\text{mol/min per mg} \)); squares, 5 mM PEP added (\( K_m = 0.10 \) mM and \( V_{max} = 0.894 \mu\text{mol/min per mg} \)).
various concentrations of threo-ds-isocitrate, and 4 mM phenylhydrazine-HCl in the absence and presence of PEP. PEP inhibited isocitrate lyase (Fig. 3A), and the Hofstee plot revealed that the inhibition was of the uncompetitive type with the $K_i$ value for PEP of 0.893 ± 0.097 mM (Fig. 3B). The inhibition constants of isocitrate dehydrogenase and isocitrate lyase for PEP are within the intracellular PEP concentration range from 0.2 to 1.0 mM, depending on carbon sources (6), suggesting that inhibition by PEP is physiologically relevant.

The $K_m$ value of NADP-isocitrate dehydrogenase for isocitrate ($K_m = 0.029$ mM) is much lower than that of isocitrate lyase ($K_m = 0.89$ mM). Isocitrate dehydrogenase is, thus, less sensitive to the availability of isocitrate, as the enzyme operates largely in the zero-order region (i.e., the concentration of isocitrate is greater than the $K_m$), but the glyoxylate pathway operates at low flux in E. coli cells, in which isocitrate concentration is below the $K_m$ of isocitrate lyase. Flux of the glyoxylate shunt largely depends on the inactivation of isocitrate dehydrogenase catalyzed by isocitrate dehydrogenase kinase/phosphatase (12) and the stimulation of isocitrate lyase (3). Inactivation of isocitrate dehydrogenase causes the possible enhancement of isocitrate lyase by increasing isocitrate concentrations. For example, when the pck gene encoding phosphoenolpyruvate carboxykinase is deleted, isocitrate lyase increased the activity of isocitrate dehydrogenase twofold, which decreases the activity to 16 to 17% of the wild-type cells (13), and thus, a larger portion of isocitrate is expected to be metabolized through the glyoxylate shunt. However, the glyoxylate shunt activity is only one-third of the flux of the isocitrate dehydrogenase pathway in the pck deletion mutant (13).

PEP may participate in the control of glyoxylate bypass and the TCA cycle. Increased PEP concentrations in pck knockout cells cause inhibition of NADP-isocitrate dehydrogenase, but the resulting accumulation of isocitrate can relieve the enzyme from allosteric inhibition by PEP. On the other hand, isocitrate lyase is subject to uncompetitive inhibition by PEP, and an increase in the concentration of the substrate, isocitrate, will further enhance the inhibition by PEP, because the inhibitor binds only to the enzyme-substrate complex. Lower flux of glyoxylate shunt, in spite of increased induction of isocitrate lyase in cells with higher PEP concentrations, can be explained by the differential inhibitory effects of PEP on isocitrate dehydrogenase and isocitrate lyase, as well as the inhibition of isocitrate dehydrogenase kinase that protects isocitrate dehydrogenase from inactivation (9).

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