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Regulation of Fructose 1,6-Bisphosphatase by Histidine under Gluconeogenic Conditions
(rabbit liver/fasting/amino-acid levels/proteolytic modification/chelating metabolites)

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Contributed by B. L. Horecker, February 21, 1974

ABSTRACT Fructose 1,6-bisphosphatase (EC 3.1.3.11) requires a free divalent metal and a metal chelate for optimum activity in the neutral pH range. The latter requirement can be satisfied by histidine, which appears to function as the chelating metabolite in vivo. Under fasting conditions, the concentrations of histidine in rabbit liver vary in the range required for activation of fructose bisphosphatase, and the enzyme itself is modified so that it becomes more responsive to histidine.

Rabbit liver and kidney fructose 1,6-bisphosphatases (Fru-P\(_2\)ase) (EC 3.1.3.11; \(n\)-fructose-1,6-bisphosphate 1-phosphohydrolase) have been shown to require both free divalent cation (\(\text{Mg}^{2+}\) or \(\text{Mn}^{2+}\)) and an EDTA-metal chelate for optimum activity at neutral pH (1, 2). A similar requirement has been demonstrated for purified Fru-P\(_2\)ase from muscle (3), and evidence has been reported for the muscle enzyme suggesting that citrate and phosphocreatine may replace EDTA under physiological conditions (4). However, similar physiological chelating agents that would activate the liver and kidney enzymes have not yet been identified, although some years ago, Hers and Eggermont (5) reported that the activity of Fru-P\(_2\)ase in crude rat-liver extracts was increased in the neutral pH range by high concentrations of histidine or imidazole.

In the present paper we report the activation of purified rabbit-liver Fru-P\(_2\)ase with physiological concentrations of histidine, which are as effective as EDTA. The requirement for histidine becomes more pronounced under gluconeogenic conditions, because of a structural modification of Fru-P\(_2\)ase in vivo whereby a small tryptophan-containing peptide is removed from the NH\(_2\) terminus (6). Thus the primary event leading to the regulation of Fru-P\(_2\)ase in gluconeogenesis may be the observed increase in lysosomes and lysosomal proteolytic activity (6, 7); this would result in a general degradation of proteins and release of free amino acids, including histidine, and the concurrent modification of Fru-P\(_2\)ase would result in an enhanced response to this amino acid. Analysis of histidine levels in the livers of fed, fasted, and refed rabbits confirm that these levels change in a manner consistent with the requirements for gluconeogenesis.

MATERIALS AND METHODS

**Materials**

d-Fructose 1,6-bisphosphate (Na\(_4\) salt), NADP, NADH, and amino acids were purchased from Sigma Chemical Co., St. Louis, Mo. Fru-P\(_2\)ase was purified as described by Traniello et al. (1).

**Methods**

**Animals.** Adult female brown rabbits, weighing 2.5–2.8 kg, were obtained from the local Italian market and maintained on a stock diet or fasted as reported (6). Animals were killed by cervical dislocation; the livers were removed immediately and cooled in crushed ice.

**Fru-P\(_2\)ase Assay.** The enzyme was assayed spectrophotometrically at pH 7.5 in the presence of 2 mM MgCl\(_2\) and 0.1 mM EDTA as described (1). Where indicated, EDTA was omitted, and the pH was changed by addition of HCl or NaOH to the same buffer.

**Analysis for Amino Acids in Liver.** Aliquots of liver were homogenized and centrifuged at 105,000 \(\times g\) as described (6, 7). Before analysis, the 105,000 \(\times g\) supernatant solution was heated at 100° for 3 min and centrifuged to remove coagulated protein. Amino-acid analyses were carried out with a Beckman model 120B Automatic Analyzer according to Spackman et al. (8).

**RESULTS**

**Effect of EDTA and Histidine on the Activity of Purified Liver Fru-P\(_2\)ase from Fed and Fasted Rabbits.** In the presence of 0.1 mM EDTA and 0.2 mM MgCl\(_2\), the specific activity of Fru-P\(_2\)ase purified from livers of fasted rabbits was identical to that of the enzyme from fed animals, with optimum activity at pH 7.3 and a small shoulder at pH 8 (Fig. 1). In the absence of EDTA, the pH optimum was shifted to 8.0 in each case, but now the specific activities of the preparations from fasted animals were significantly lower.

Histidine was as effective as EDTA in activating the enzyme preparations from fed rabbits and from rabbits fasted for 36 and 96 hr. With preparations from fasted animals, optimum activity with histidine was observed at pH 7.5, rather than 7.3 (Fig. 1). The requirement for histidine, as for EDTA, was greater with the preparations from fasted animals. At pH 7.0, where the enzyme from fasted animals was stimulated nearly 5-fold, half-maximal activation was observed with approximately 0.3 mM histidine, and 1.0 mM was sufficient for full activation (Fig. 2).

In order to eliminate any possible effects of histidine on the coupling enzymes in the spectrophotometric assays, and to prove that histidine was not simply removing traces of heavy
metals, the enzyme preparations were dialyzed against EDTA and assayed by the liberation of Pi, using concentrations of enzyme 200-fold higher than those employed in the spectrophotometric assay. Activation by histidine was equivalent to that observed with the spectrophotometric assay, and the pH optimum was again shifted from 8 to 7.5 (Fig. 3).

Specificity of Activation by Histidine. All of the other naturally occurring amino acids were tested at 1 mM concentration, and only arginine was found to replace histidine. At pH 7.5, 1 mM arginine was 75-80% as effective as histidine. Citrulline and ornithine were also tested and found to be inactive.

Histidine Levels in Extracts of Liver from Fed and Fasted Animals. The level of histidine in rabbit liver increased markedly when the animals were fasted, and after 96 hr reached five times the fed value (Table 1). Within 12-24 hr after refeeding, the levels had returned to those found in the fed animals. Arginine could not be detected in any of the samples tested.

Evaluation of Intracellular Fru-P2ase Activity. Prolonged fasting affects the intracellular activity of Fru-P2ase in two ways, both correlated with the increased activity of lysosomal proteases. On the one hand, the enzyme itself is altered, becoming less active in the absence of chelating agents but more responsive to activation by these agents, including histidine. At the same time, increased proteolytic activity is associated with a 5-fold increase in the level of free histidine in the liver cytoplasm. From the histidine requirement for Fru-P2ase preparations isolated from livers of fed and fasted animals (Fig. 2), and the concentrations of histidine found in the livers under the same conditions, we estimated that the activity of the enzyme in vivo in these conditions would be increased by nearly 4-fold (Table 2).

![Fig. 1. Effect of pH on activities of Fru-P2ase isolated from livers of fed and fasted rabbits. Fru-P2ase activity was assayed spectrophotometrically. The buffer used throughout was 20 mM TEA-20 mM DEA, adjusted to the pH values indicated by addition of HCl. (•, ×) Results obtained with enzymes isolated from control animals; the same curves were obtained with enzymes isolated from animals fasted for 36 and 96 hr. (○) Values obtained in the absence of EDTA or histidine.](image1)

![Fig. 2. Effect of histidine concentration of Fru-P2ase activity at pH 7.0. The assays were carried out as described in Methods, except the pH was adjusted to 7.0. EDTA was omitted and histidine was added as indicated.](image2)

![Fig. 3. Effect of pH on Fru-P2ase activity measured in presence and absence of histidine at high concentrations of enzyme. Fru-P2ase, purified from liver of rabbits fasted for 36 hr, was filtered through Sephadex G-100 that had been equilibrated with 10 mM Na acetate, pH 6.5, containing 0.1 mM EDTA. Fractions containing activity were precipitated with 30% saturated (NH4)2SO4 and dialyzed 4 hr against 10 mM Na acetate, pH 6.5. The incubation mixture contained, in 1.0 ml: 0.02 ml of dialyzed enzyme solution containing 0.4 mg of protein, 1 mM Fru-P2, 2 mM MgCl2, 20 mM TEA-20 mM DEA at the pH indicated, and 1 mM histidine where indicated. Aliquots of 0.1 ml were removed at 0, 1, 2, 3, and 7 min and analyzed for inorganic phosphate (9). The specific activity, measured at 0°, was 15-fold less than in the spectrophotometric assay at 25°.](image3)
**Table 2. Calculated Fru-P2ase activities in rabbit liver under various conditions of fasting and refeeding**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Wt. of rabbit (g)</th>
<th>Wt. of liver (g)</th>
<th>With EDTA</th>
<th>Without EDTA</th>
<th>Calculated†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed</td>
<td>2700 ± 100</td>
<td>80 ± 7</td>
<td>3.8</td>
<td>1.8</td>
<td>2.1</td>
</tr>
<tr>
<td>Fasted, 36 hr</td>
<td>2660 ± 90</td>
<td>75 ± 8</td>
<td>2.0</td>
<td>0.8</td>
<td>1.6</td>
</tr>
<tr>
<td>Fasted, 96 hr</td>
<td>2150 ± 100</td>
<td>55 ± 6</td>
<td>8.0</td>
<td>2.7</td>
<td>8.0</td>
</tr>
<tr>
<td>Refed, 6 hr</td>
<td>2200 ± 50</td>
<td>60 ± 5</td>
<td>5.3</td>
<td>—</td>
<td>4.1</td>
</tr>
<tr>
<td>Refed, 12 hr</td>
<td>2200 ± 80</td>
<td>60 ± 8</td>
<td>4.6</td>
<td>—</td>
<td>2.7</td>
</tr>
<tr>
<td>Refed, 24 hr</td>
<td>2300 ± 70</td>
<td>65 ± 5</td>
<td>4.0</td>
<td>—</td>
<td>2.0</td>
</tr>
</tbody>
</table>

* The animals were kept as described (6). Four to 13 animals were used in each experiment.
† The data were taken from Pontremoli et al. (6).

An interesting consequence of the histidine requirement for the modified Fru-P2ase is a sharp decrease in intracellular activity on refeeding, due to the decrease in histidine levels. These levels fall to one-half and one-quarter of the fasted levels in 12 and 24 hr, respectively (Table 2).

**DISCUSSION**

Hers and his coworkers (5, 10) were the first to demonstrate activation of Fru-P2ase in muscle and liver extracts by histidine or imidazole buffers, and it was suggested by McGilvery (11) that physiological regulation of Fru-P2ase activity might involve changes in the concentration of chelating metabolites. Although it has become customary to add EDTA to the assay system for Fru-P2ase, it was only recently established that the enzymes purified from muscle (3), liver (1), and kidney (2) require both a divalent cation and a metal chelate for optimum activity in the neutral pH range.

We have now obtained evidence for a role of histidine as the chelating metabolite that regulates the activity of liver Fru-P2ase in the cell. The effect of histidine is highly specific: of all the other amino acids tested only arginine was effective, and in confirmation of earlier reports (12, 13) we found the levels of arginine in liver to be extremely low in both fed and fasted animals. On the other hand, the concentrations of histidine in the liver cell under fed and fasting conditions range from 0.2 to 1.0 mM, precisely in the range in which the enzyme is activated (see Fig. 2). In addition to these changes in histidine levels, the properties of the enzyme itself are modified under gluconeogenic conditions, so that it becomes more responsive to histidine than the enzyme isolated from the livers of fed animals (see Fig. 1). Thus, in the livers and kidneys of fasted rabbits three factors serve to enhance the activity of Fru-P2ase: (i) the total enzyme activity increases by 2-fold (6), (ii) the enzyme becomes more responsive to histidine, and (iii) the levels of histidine are increased by nearly 5-fold. The last two are clearly correlated with the increase in proteolytic activity, particularly "free" proteolytic activity, reported in livers and kidneys of fasted animals (6, 7).

Other factors will undoubtedly be shown to regulate the activity of this enzyme in vivo. Inhibition of AMP is a general property of Fru-P2ases (14-18), although the levels of AMP do not appear to change significantly in fasted or refed animals (19). Instead, regulation may involve proteolytic modification of the enzyme to a form less sensitive to inhibition by AMP. Evidence for loss of AMP sensitivity during proteolytic modification of purified liver FDPase has been reported (6, 20, 21), but this modification has not yet been shown to occur in vivo. Rabbit-liver Fru-P2ase has also been shown to be activated by fatty acids, especially oleic acid (22), and it will be of interest to determine the effects of proteolytic modification in vitro and in vivo on this activation.

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