NEUTRAL LIPID ACCUMULATION IN YEAST DUE TO INOSITOL DEFICIENCY

KINETIC STUDIES ON THE RECIPROCAL REGULATION BY FRUCTOSE BISPHOSPHATE AND CITRATE OF YEAST ACETYL CoA CARBOXYLASE

Takako Tomita, Ryuichi Hasegawa, and Eiichi Hayashi

Department of Pharmacology, Shizuoka College of Pharmaceutical Sciences, 2-2-1, Oshika, Shizuoka 422, Japan
(Received December 28, 1978)

Summary Neutral lipids, especially triacylglycerols, accumulated due to myo-inositol deficiency both in the cells of Saccharomyces carlsbergensis (Hayashi et al. (1976) J. Biol. Chem., 251, 5759–5769) and in the liver of the rat (Hayashi et al. (1974) Biochim. Biophys. Acta, 360, 134–155). The accumulation of triacylglycerols in the deficient yeast resulted, at least partly, from an enhancement of acetyl CoA carboxylase activity. The activation of the enzyme reflected the fluctuation due to the deficiency in the levels of fructose bisphosphate and citrate (Hayashi et al. (1978) Biochim. Biophys. Acta, 540, 231–237). Thus, the kinetics of the regulation of acetyl CoA carboxylase by these intermediates was studied. In physiological concentrations fructose bisphosphate sigmoidally activated acetyl CoA carboxylase from yeast with the Hill coefficient of 3, while citrate counteracted the fructose bisphosphate activation in a sigmoidal manner with the Hill coefficient of 2. Fructose bisphosphate markedly increased the apparent $V_{\text{max}}$ value of acetyl CoA carboxylase for the substrate, ATP and slightly decreased the apparent $K_m$ value. Citrate greatly decreased the apparent $V_{\text{max}}$ value increased by fructose bisphosphate.

Keywords acetyl CoA carboxylase, Saccharomyces carlsbergensis, citrate, fructose bisphosphate, $V_{\text{max}}$, $K_m$, myo-inositol deficiency, neutral lipid accumulation

In the course of our investigation on the abnormal lipid accumulation in myo-inositol deficient Saccharomyces carlsbergensis (S. carls.), it was found that acetyl CoA carboxylase [EC 6.4.1.2], a rate-limiting enzyme of fatty acid synthesis, from this yeast, was activated by fructose bisphosphate and the activation was counteracted by citrate at the physiological concentration (1).

1富田多嘉子，長谷川隆一，林栄一
Further, it has been proven that such a dual control of the acetyl CoA carboxylase with fructose bisphosphate and citrate is physiologically functioning \textit{in vivo} in this yeast \cite{1}. A marked elevation of fructose bisphosphate level and a drastic decrease of citrate level due to \textit{myo}-inositol deficiency induced a great activation of acetyl CoA carboxylase in this yeast.

Fructose bisphosphate has been reported to exert stimulatory effects on several enzymes from various sources: fatty acid synthetase from pigeon liver \cite{2}; glycogen synthetase in \textit{E. coli} \cite{3}; phosphofructokinase from rat skeletal muscle \cite{4}; pyruvate kinase from \textit{E. coli} \cite{5}, \textit{S. cerevisiae} \cite{6}, the liver of the rabbit \cite{7} and the rat \cite{8}; phosphoenolpyruvate carboxylase from \textit{E. coli} \cite{9}; lactate dehydrogenase from \textit{Butyrivibrio fibrisolvens} \cite{10} and \textit{Streptococcus lactis} \cite{11}. Citrate activates mammalian acetyl CoA carboxylase by polymerizing the molecule \cite{12} and controls the metabolism of pyruvate by interconverting pyruvate dehydrogenase between its active and inactive forms \cite{13}, while citrate inhibits phosphofructokinase \cite{4}, phosphoenolpyruvate carboxylase \cite{9}, pyruvate kinase \cite{14} etc.

As both the intermediates play important roles in the regulation of several enzymes, their levels are finely controlled by rate-limiting enzymes such as phosphofructokinase, fructose bisphosphatase, ATP-citrate lyase and citrate synthetase. The mechanism by which the levels of fructose bisphosphate and citrate fluctuate due to \textit{myo}-inositol deficiency in the yeast, has been described elsewhere \cite{15}. This paper deals with the change of kinetic parameters of acetyl CoA carboxylase from normal \textit{S. carls} by the presence of fructose bisphosphate and citrate.

\section*{METHODS}

\textit{Saccharomyces carlsbergensis} 4228 (ATCC 9080) was cultivated for 42 hr in \textit{myo}-inositol-supplemented medium \cite{1}. After the cells had been harvested by refrigerated centrifugation (3,000 rpm, 10 min), about 6 g of the cells (wet weight) were homogenized by a Braun homogenizer for 30 sec together with 10 g glass beads (0.45–0.50 φ) in 10 ml of 0.6 M mannitol phosphate buffer (pH 7.2). The 100,000 g supernatant of the homogenate was submitted to gel-filtration with a Sephadex G-25 column. The gel-filtrate was used as the acetyl CoA carboxylase enzyme preparation in this experiment. The assay procedure for acetyl CoA carboxylase has been described in the previous paper \cite{1}.

\section*{RESULTS}

\textit{The activation of acetyl CoA carboxylase by fructose bisphosphate and the counteraction by citrate of the activation}

As Fig. 1(a) shows, fructose bisphosphate strongly stimulated the acetyl CoA carboxylase in a sigmoidal manner over the physiological concentration. The level of fructose bisphosphate in \textit{S. carls} was in the range of 0.68±0.018 (mM) and rose to about 1.8 (mM) due to \textit{myo}-inositol deficiency.
An addition of 0.5, 1.0 and 2 mM of fructose bisphosphate increased the acetyl CoA carboxylase activity 1.2-, 3- and 10-fold, respectively, and the activation reached a maximum at 5 mM fructose bisphosphate. The data in Fig. 1(a) were analyzed according to the empirical Hill equation (Equation 1),

\[
\log_{10} \frac{V}{V_{\text{max}} - V} = n \log_{10} [\text{fructose bisphosphate}] - \log_{10} K
\]

and the result of analysis is shown in Fig. 1(b). In Equation (1), \(n\) is the Hill coefficient which defines the extent of interaction between multiple interdependent sites, and \(K\) is a complex constant. The Hill coefficient of fructose bisphosphate activation is 3.1 for the slope of the linear equation calculated using the least square method.

Citrate, which stimulates mammalian acetyl CoA carboxylase, neither activated nor inhibited the yeast acetyl CoA carboxylase. This result coincides with the result obtained by Lynen et al. (16). However, citrate counteracted fructose bisphosphate activation at a physiological concentration, as shown in Fig. 2(a). The acetyl CoA carboxylase activity in the presence of fructose bisphosphate (1 mM) sigmoidally decreased as the citrate concentration increased. In the absence of fructose bisphosphate, however, citrate exerted no effect on acetyl CoA carboxylase. The citrate level in \(S. \text{carls.}\) ranged around 11.92 ± 0.296 (mM) and the level was reduced to about 4.5 mM due to \textit{myo}-inositol deficiency. The data in Fig. 2(a) are plotted in Fig. 2(b) according to the empirical Hill equation for changes in velocity in the presence of the inhibitor,

\[
\log_{10} \frac{V_0 - V_i}{V_i - V_{\text{sat}}} = n \log_{10} [\text{citrate}] - \log_{10} K
\]

In Equation 2, \(n\) and \(K\) have the same meaning as in Equation 1. \(V_i\) and \(V_0\) are the initial velocities observed in the presence or absence of a given concentration of citrate, while \(V_{\text{sat}}\) is the velocity observed in the presence of a saturating

![Graph of acetyl CoA carboxylase activity as a function of fructose bisphosphate.](image)
Fig. 2. Counteraction by citrate of fructose bisphosphate activation of acetyl CoA carboxylase. (a), Acetyl CoA carboxylase activity as a function of citrate concentration in the presence of 1 mM fructose bisphosphate; (b), the data in (a) are plotted according to the empirical Hill equation 2 (See the text).

Fig. 3. The inhibition of acetyl CoA carboxylase by citrate at changing fixed concentrations of fructose bisphosphate. In this figure, the data are plotted according to the empirical Hill equation 2. Fructose bisphosphate 0.5 mM (○), 1.0 mM (■), 1.5 mM (▲), 2.0 mM (●).

The changes in properties of acetyl CoA carboxylase for ATP by fructose bisphosphate and citrate

Figure 4 represents acetyl CoA carboxylase activity as a function of substrate concentration of the metabolite. The Hill coefficient for citrate is 2.2. The dependence of the Hill coefficient for citrate on fructose bisphosphate concentration is shown in Fig. 3. The Hill coefficient for citrate was 2.4, 2.3, 2.1 and 1.8, respectively, in the presence of 0.5, 1.0, 1.5 and 2.0 mM fructose bisphosphate.
Table 1. Changes by fructose bisphosphate of the $K_m$ and $V_{max}$ of acetyl CoA carboxylase for ATP.

<table>
<thead>
<tr>
<th>Fructose bisphosphate (mM)</th>
<th>$K_m$ (µM)</th>
<th>$V_{max}$ ($10^{-3} \times$ dpm/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>494</td>
<td>2.12</td>
</tr>
<tr>
<td>0.5</td>
<td>440</td>
<td>4.52</td>
</tr>
<tr>
<td>1.0</td>
<td>320</td>
<td>15.3</td>
</tr>
<tr>
<td>1.5</td>
<td>265</td>
<td>27.0</td>
</tr>
<tr>
<td>2.0</td>
<td>287</td>
<td>33.4</td>
</tr>
</tbody>
</table>
Table 2. Changes by citrate of the $K_m$ and $V_{max}$ of acetyl CoA carboxylase for ATP in the presence of fructose bisphosphate.

<table>
<thead>
<tr>
<th>Concentration (mM) of</th>
<th>$K_m$ (µM)</th>
<th>$V_{max}$ ($10^{-3} \times$ dpm/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose bisphosphate</td>
<td>Citrate</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>595</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>517</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>280</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>327</td>
</tr>
</tbody>
</table>

ATP at changing fixed concentrations of fructose bisphosphate. The reaction proceeds according to the Michaelis-Menten equation. Apparent $K_m$ and $V_{max}$ values for ATP were obtained from Lineweaver-Burk plots of the data in Fig. 4 (Table 1). As the concentration of fructose bisphosphate increased (0 to 2 mM), the $K_m$ value of acetyl CoA carboxylase for ATP slightly decreased, while the $V_{max}$ markedly increased. This result indicates that the activation of acetyl CoA carboxylase by fructose bisphosphate is mainly ascribed to an increase in the maximum reaction velocity and partly to a decrease of $K_m$ value.

Figure 5 shows the inhibitory effect of citrate on acetyl CoA carboxylase as a function of ATP concentration in the presence of 1 mM fructose bisphosphate. The inhibitory effect on fructose bisphosphate-activated acetyl CoA carboxylase was greater at higher concentrations than at low concentrations of ATP. The $K_m$ and $V_{max}$ values of acetyl CoA carboxylase for ATP in the presence of citrate and fructose bisphosphate were calculated from Lineweaver-Burk plots from the data in Fig. 5 (Table 2). In the presence of 1 mM fructose bisphosphate, citrate markedly decreased the $V_{max}$ for ATP. It also slightly affected the affinity. The result suggests that citrate counteracts fructose bisphosphate activation of acetyl CoA carboxylase primarily by decreasing the $V_{max}$ which is increased by fructose bisphosphate.

**DISCUSSION**

The rate of some enzyme-catalyzed reactions increases in a hyperbolic curve with an increase in concentration of the substrate, while in others the initial velocity increases sigmoidally. This results from the substrate binding at more than one site on the enzyme protein (17). Regulators often bind at more than one site of the enzyme, thereby changing the reaction velocity (18). Kinetic parameters (maximum reaction velocity; $V_{max}$, affinity of the enzyme for the substrate; $K_m$, and Hill coefficient; $n$) change in the presence of regulators for the reaction. Thus, the mode of interaction of the enzyme with the regulators is classified into 5 types by the changes in the parameters; changes 1) in only $K_m$, 2) in only $V_{max}$, 3) changes in $K_m$ and $V_{max}$, 4) in $K_m$ and $n$, 5) in $K_m$, $V_{max}$ and $n$ together.
Pyruvate carboxylase from the liver of birds (18, 19), mammals (18) and invertebrates (20), requires acetyl CoA for activation. A sigmoidal relationship was observed between the initial velocity and acetyl CoA concentration. α-Ketoglutarate is a specific inhibitor for pyruvate carboxylase from chicken liver (18). Addition of α-ketoglutarate causes the apparent $K_A$ for acetyl CoA to become less favorable but does not affect the apparent $V_{max}$. The Hill coefficient for acetyl CoA activation is 3, but that for α-ketoglutarate inhibition varies with acetyl CoA concentration. Pyruvate carboxylase of certain microbial and fungal origins is subject to regulatory inhibition by L-aspartate, thus providing a counterbalance to activation by acetyl CoA (20).

Phosphoenolpyruvate carboxylase from E. coli (9) is activated by fructose bisphosphate at low concentrations synergistically with either acetyl CoA or laurate. The Hill coefficient for fructose bisphosphate activation is 1. Activation with each of the three activators is markedly inhibited by aspartate, and to a lesser extent, by citrate. The inhibition is competitive.

Pyruvate kinase from the liver of the rat (7), the rabbit (8) and the chicken (9) as well as yeast (6) is also allosterically activated by fructose bisphosphate and inhibited by citrate. The Hill coefficients of yeast pyruvate kinase for fructose bisphosphate activation and citrate inhibition are 2.7 and 3, respectively. Fructose bisphosphate markedly decreases the $K_m$ value for substrate phosphoenolpyruvate, transforming the phosphoenolpyruvate characteristic to a hyperbolic curvature.

From the Hill coefficients of yeast acetyl CoA carboxylase for fructose bisphosphate and citrate, acetyl CoA carboxylase seems to bind with fructose bisphosphate at 3 sites, while citrate binds at 2 sites independent of fructose bisphosphate concentrations, suggesting that fructose bisphosphate and citrate interact at different sites on the enzyme. Activation of yeast acetyl CoA carboxylase by fructose bisphosphate is primarily due to a large change in the maximum velocity, and partly due to a change in the affinity of the enzyme for the substrate ATP. Citrate is an allosteric potent inhibitor at physiological concentrations. It affects the maximum velocity and has little effect on the affinity of the enzyme for ATP and fructose bisphosphate.

REFERENCES


