A STABILIZING FACTOR FOR LIVER PHOSPHOFRUCTOKINASE

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SUMMARY. A labile, non-dialyzable factor is present in rat liver supernatant fluid which protects liver phosphofructokinase against thermal inactivation. The factor is not present in other tissues examined, except kidney, and does not affect the stability of other hepatic enzymes tested (glucokinase and pyruvate kinase). The level of the factor is decreased in starvation, where the in vivo degradation rate of phosphofructokinase is increased, and restored with refeeding. The level of factor and of phosphofructokinase also change in parallel with insulin deprivation and administration. These findings suggest that regulation of phosphofructokinase levels in the liver is mediated by alterations in the content of the protective factor.

Dunaway and Weber have recently discovered that the level of the major hepatic isozyme of phosphofructokinase, PFK-L_2, is reduced in diabetes and starvation, and have demonstrated in the case of the latter that this change is a result of an increased degradation rate of the enzyme[1]. Very little is known of the nature of the protein turnover process and its regulation, in spite of the fact that a number of examples now exist of modulation of enzyme levels through alterations in their degradation rates, as in this case (for review, see (1)). It is not even known, for example, whether thermal lability or proteolytic susceptibility is the rate-determining factor, or whether, as seems likely, one process is underlying in some cases and the other in others. The finding of a specific protective factor for PFK-L_2 with its apparent relevance to the regulation of PFK-L_2 degradation rates in vivo offers a potentially useful tool for obtaining insight into the turnover process.

METHODS

PFK-L_2 was isolated from rats by the method of Dunaway and Weber[1] and was assayed as previously described (2). The final preparations were in a

[1] To be published elsewhere.

solution of 50 mM Tris-Cl, pH 8.0, 50 mM NaF, 10 mM dithiothreitol, and 1.0 mM ATP, and contained about 200 units/ml of specific activity 50 to 70.

Liver supernatant fluid as a source of the protective factor was obtained by homogenization of rat liver at 0° in 2 volumes of 50 mM Tris-Cl, pH 8.4, followed by centrifugation at 100,000 x g for 45 min. Low molecular weight compounds were removed by passage of 5 ml of the supernatant solution through a column (1.5 cm x 15 cm) of Sephadex G-25 (coarse) equilibrated with the homogenizing solution. A unit of protective factor is defined as the amount which extends by 1 min the half-life of PFK-L_2 during the inactivation reaction (see Appendix).

Partially purified preparations of rat liver glucokinase and pyruvate kinase L were obtained by methods adapted from published procedures (3,4).

RESULTS

Fig. 1 shows the time course of inactivation of PFK-L_2 in the absence and presence of protective factor. Proportionality of the half-time extension to the amount of factor is demonstrated in Fig. 2.

Table I contains the results of tests of the ability of extracts of other rat tissues and a non-specific protein to protect PFK-L_2. As can be seen only liver and kidney possessed significant protective ability. Table II demonstrates the independence of the effect on PFK-L_2 concentration as predicted from the model (see Appendix) and the lack of protective effect on liver glucokinase and pyruvate kinase L (the isozymes which, like PFK-L_2, are the ones responsive to nutritional and hormonal state (5,6).

The lability of the factor and the ability of glucose to stabilize it are shown in Table III.

Table IV shows the effects on protective factor levels of fasting and refeeding and of diabetes and insulin treatment. With both fasting and diabetes there was a very marked reduction in protective factor levels which was reversed by refeeding or insulin treatment, respectively. The reversal in both cases was rapid, overshooting normal levels on the first day before returning to control values.

DISCUSSION

The results presented here demonstrate the existence of a factor in liver and kidney which protects the major liver isozyme of phosphofructokinase

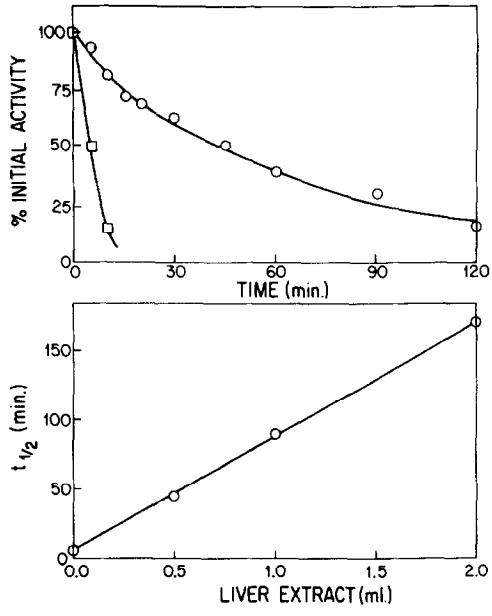

Fig. 1. Thermal inactivation of PFK-L_2. Incubations were at 37° with 20 µl of PFK-L_2 solution plus 2.0 ml of homogenizing buffer (pH 8.0 at this temperature) (squares), or 1.0 ml of homogenizing buffer plus 1.0 ml of liver supernatant fluid (20.3 mg protein/ml) prepared as described in the text (circles).

Fig. 2. Proportionality of PFK-L_2 half-life ($t_{\frac{1}{2}}$) to concentration of protective factor. The experiment was carried out as in Fig. 1 with various volumes of liver supernatant fluid (30.6 mg protein/ml) added in place of buffer.

(PFK-L_2) against thermal inactivation. This factor is evidently a protein as indicated by its size and lability, but this remains to be conclusively demonstrated by isolation. The lack of protective effect toward the other enzymes tested and the correspondence in tissue distribution of the factor and the PFK-L_2 isozyme[1] permit the tentative conclusion that the factor is specific for this enzyme. This is consistent with other evidence that only the PFK-L_2 isozyme is subject to regulatory control while the minor isozyme of liver, PFK-L_1, is not[1].

The correlation between changes in the level of the protective factor and of PFK-L_2 in altered nutritional and hormonal states and the time relationships thereof suggest a close interdependency between the two. Since the decline and restoration of PFK-L_2 levels, at least in the case of fasting and re-

Table I

Tissue Distribution of PFK-L_2 Protective Factor

Incubations were as described in Fig. 1 with 16 mg of protein from the tissue indicated. Tissue extracts were prepared as described in the text for liver.

Tissue	Protective factor (units)
liver	31
kidney	25
brain	12
heart	11
muscle	8
serum	4
testis	4
serum albumin (bovine)	5

feeding, have been shown to reflect changes in the degradation rates of the enzyme[1] it can be proposed that the protective factor plays a direct role in determining the stability of the enzyme in vivo.

APPENDIX

That the half-life increase is linearly proportional to the amount of protective factor is deduced as follows.

The total enzyme (E_t) consists of free enzyme (E_f) and enzyme bound to the protective factor (EP),

i.e., $(E_t) = (E_f) + (EP)$.

Table II

Specificity of PFK-L_2 Protective Factor

Incubations were as described in Fig. 1. The liver extract contained 22 mg of protein/ml.

Enzyme	Half-lives (min)	
	control	+ 2.0 ml liver extract
PFK-L_2 (1.25 units)	5	57
PFK-L_2 (2.5 units)	5	58
PFK-L_2 (5.0 units)	5	56
glucokinase (1.8 units)	3	3.5
pyruvate kinase L (1.2 units)	3	3

It is the rate of decrease of total enzyme that is measured, i.e., $d(E_t)/dt$. Assuming that EP is not inactivated at a significant rate, then,

$$\frac{d(E_t)}{dt} = k(E_f).$$

Let $\frac{(EP)}{(E_t)} = r$. Then, $(E_f) = (E_t)\ (1-r)$.

Therefore, $\frac{d(E_t)}{dt} = k(1-r)\ (E_t)$.

$k(1-r)$ is the experimentally determined inactivation rate constant, which we will call k', i.e., $k' = k(1-r)$.

It now remains to determine r as a function of protective factor concentration (P).

The reaction is,

Table III

Lability of the Protective Factor and its Stabilization by Glucose

Incubations were as described in Fig. 1 with 1.0 ml of liver supernatant fluid (21.5 mg protein). Glucose, when added was at a concentration of 0.25 M. Glucose itself had no effect on the half-life of PFK-L_2.

Conditions of storage	Protective activity (% of initial[a])	
	time of storage	
	24 hr	48 hr
4°, no glucose	0	0
4°, + glucose	100	60
-20°, no glucose	10	0
-20°, + glucose	100	72
liq. N, no glucose	20	0
liq. N, + glucose	100	72

[a] The initial activity of the fresh supernatant fluid was 25 units/ml.

$$E_f + P_f \longrightarrow EP,$$

$$\text{Define } K_d = \frac{(E_f)\ (P_f)}{(EP)} = \frac{(E_t - EP)\ (P_t - EP)}{EP}$$

Assume $(P) \gg (E)$

$$\text{Then, } \frac{(P_t)}{K_d + (P_t)} = \frac{(EP)}{(E_t)} = r$$

$$k' = \left[1 - \frac{(P_t)}{K_d + (P_t)}\right] \cdot k = \frac{K_d}{K_d + (P_t)} \cdot k$$

Let $t_{\frac{1}{2}}$ be the experimentally determined half-life.

Table IV

Effect on Liver Protective Factor Levels of Fasting and Refeeding and of Diabetes and Insulin Treatment

Data are averages of 2 animals. Refeeding began after 6 days of fasting. Rats were made diabetic by the injection (i.p.) of 200 mg alloxan monohydrate per kg body weight after a 30 hr fast and were used 7 days later. Insulin treatment was 2 units/day (s.c.) of protamine zinc insulin (Lilly). Blood sugar levels of the diabetic rats were between 500 and 550 mg per 100 ml. Incubations were as described in Fig. 1 with 1.0 ml of liver supernatant fluid.

Treatment	Protective factor	DNA
	% of control[a]	mg/g liver
controls	100	1.9
3 day fast	41	3.1
6 day fast	12	4.0
1 day refeed	140	2.5
2 day refeed	79	2.1
3 day refeed	91	1.9
diabetic	26	2.8
1 day insulin	180	2.2
2 day insulin	116	1.7
3 day insulin	89	1.9

[a] The control value was 91 units/mg DNA.

$$\text{Then, } t_{\frac{1}{2}} = \frac{0.69}{k'} = \frac{0.69}{k\left[\frac{K_d}{K_d + (P_t)}\right]} = \frac{0.69}{k} + \frac{0.69}{k} \cdot \frac{(P_t)}{K_d} .$$

Thus $t_{\frac{1}{2}}$ is proportional to the factor concentration. It may also be

noted that $t_{\frac{1}{2}}$ is independent of enzyme concentration. The last equation may also obviously be utilized to obtain the dissociation constant, K_d, from a plot of $t_{\frac{1}{2}}$ vs. (P_t).

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