ALTERATION OF ENZYMATIC ACTIVITIES OF RAT LIVER DIPHOSPHATASE BY TREATMENT WITH PAPAIN OR UREA

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We have previously reported that a highly purified preparation of fructose-1, 6-diphosphatase (FDPase) from rat liver will also catalyze the hydrolysis of sedoheptulose-1, 7-diphosphate (SDP) to sedoheptulose-7-phosphate (S7P) (1). This appears to be the specific FDPase originally described by Gomori (2), and more recently studied by Pogell (3) and McGilvery (4), but its activity with SDP had not previously been reported. Evidence has been presented (1) which strongly supports the conclusion that a single enzyme is responsible for activity with both FDP and SDP.

We have now found that the enzyme protein can be modified by treatment with papain or urea in such a manner as to change the relative activities toward the two substrates. On the basis of the kinetic properties of the modified protein, however, it would still appear that a single enzyme is responsible for the two activities.

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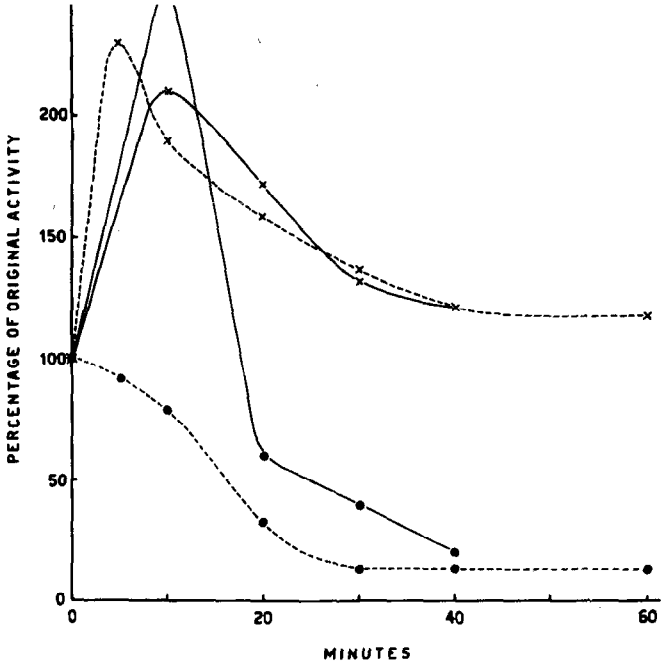
The enzyme purification and assays were carried out as previously described (1) and were based on the liberation of inorganic phosphate from FDP and SDP. For the kinetic studies, FDPase activity was followed by the appearance of F6P. Papain was a recrystallized commercial preparation from Sigma Chemical Company. Substrates were purchased or prepared as previously indicated (1).

Digestion with papain at a ratio of 20:1 produced marked changes in the enzyme activities. At 26°, both FDPase and SDPase activities increased markedly, reaching levels approximately two-fold greater than the original activity. With longer digestion with papain the activity toward FDP returned to nearly the original level, while that toward SDP was reduced to very low levels. Similar results were obtained when the enzyme was incubated with papain at higher temperature (37°) except that the early increase in activity with SDP was lacking. In each case the final result was a change in ratio of SDPase to FDPase activities from about 1.0 to 0.1. (Fig. 1).

Similar changes to those observed with papain at 37° occurred when the enzyme was treated with increasing concentrations of urea (Fig. 2). The activity with FDP was activated at low concentration of urea (0.5M) and then returned to approximately the original value as the concentration of urea was increased to about 1 M. The activity with SDP in 1 M urea was reduced to about 10% of the original. Again the modified enzyme showed a ratio of SDPase to FDPase activities of 0.1. The time of treatment with urea (30 minutes) was sufficient to stabilize the enzyme activity at the levels shown in the figure.

The effect of urea was found to be reversible. When the urea-treated enzyme was diluted in the absence of urea the activities returned to es-



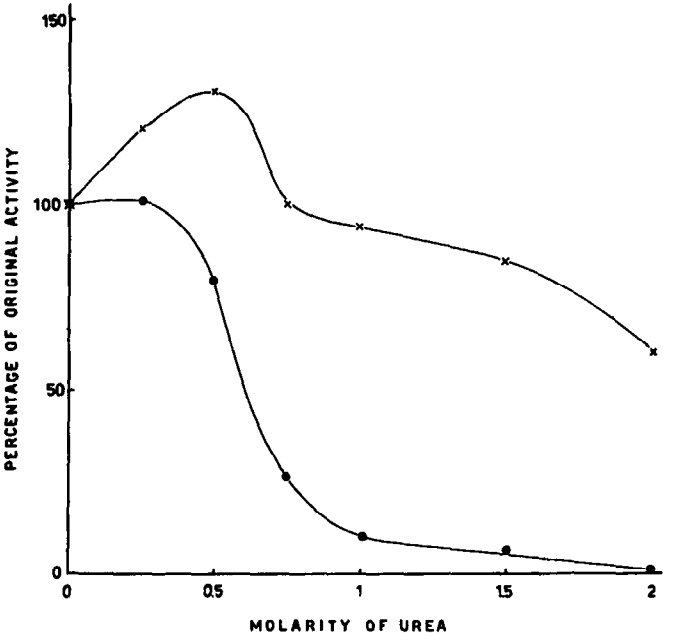


Fig. 1. Effect of papain digestion on FDPase and SDPase activities. A purified preparation of diphosphatase (original activity = 740 units of FDPase and 770 units of SDPase per mg of protein) was incubated in the presence of papain at 37° (dotted lines) and at 26° (full lines.) The incubation mixture (0.15 ml) contained: acetate buffer, 8×10^{-2}M, pH 5.5; cysteine, 5×10^{-3}M; versene, 3×10^{-3}M; papain, 15 γ/ml, and diphosphatase, 0.3mg/ml. Aliquots were collected and analyzed for FDPase and SDPase activities. Solid circles represent activities with SDP; x's represent activities with FDP.

Fig. 2. Effect of urea treatment on diphosphatase activity. Aliquots of the enzyme preparation were incubated in the presence of different concentrations of urea for 30 minutes at 26° and then assayed for FDPase (x) and SDPase (•) activities. The incubation mixtures (0.5 ml) contained: TEA buffer, 0.1 M, pH 9.0; urea at the indicated concentrations, and enzyme 15 γ. The assays were carried out in the same mixture by adding FDP, 1×10^{-3}M and $MnCl_2$, 1×10^{-4}M, or SDP, 1×10^{-3}M and $MnCl_2$ 3×10^{-3}M.

sentially the original values and the ratio of activities with SDP and FDP was again 1.0. This reversal required incubation for 10-20 minutes at 26°.

Kinetic studies with the modified enzyme protein support the previous view that a single protein is responsible for the two enzymic activities. These studies were carried out with the urea-treated enzyme which possessed an activity ratio of 0.1. The K_s for FDP was found to be identical with that of the untreated enzyme (Table 1). The K_i for SDP with the modified enzyme, calculated from its effect on the hydrolysis of FDP, was identical with the K_s determined with the untreated enzyme. We have previously reported (1) that with the native enzyme, identical constants are obtained for SDP when it is employed as a substrate or as a competitive inhibitor of the hydrolysis of FDP.

Table I

Michaelis-Menten Constants of the

Native and Modified Enzyme Protein

Constant	Native Enzyme	Urea Treated
K_s (FDP)	0.85×10^{-5} M	0.90×10^{-5} M
K_s (SDP)	0.31×10^{-4} M	-----
K_i (SDP)	0.34×10^{-4} M	0.32×10^{-4} M

Further studies are in progress to determine the nature of the changes in the enzyme protein produced by digestion with papain or by treatment with urea. It is of particular interest that the affinities for the substrates

appear to be unchanged despite the marked changes in enzyme activities. The enzyme appears to possess a unique binding site for FDP and SDP but to be capable of being modified differentially with respect to its catalitic activities. Since FDPase is a key enzyme in the biosynthesis of hexose, these changes suggest that it may be a site for a metabolic control mechanism.

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REFERENCES

1. Bonsignore, A.; Pontremoli, S., Mangiarotti, G., Mangiarotti, M., and De Flora, A., J. Biol. Chem., in press.
2. Gomori, G., J. Biol. Chem., 148, 139 (1943).
3. Pogell, B. M., and McGilvery, R. W., J. Biol. Chem., 208, 149 (1954).
4. Mokrasch, L. C., and McGilvery, R. W., J. Biol. Chem., 221, 909 (1956).