INACTIVATION OF MICROSOMAL GLUCOSE-6-PHOSPHATASE BY PYRIDOXAL-5'- PHOSPHATE

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Received February 16, 1971

Summary. Glucose-6-phosphatase activity was inactivated when liver microsomes were incubated with pyridoxal-5'-phosphate at 30° in 0.05 M Tris-buffer, pH 7.0. Addition of glucose-6-phosphate completely protected the enzyme from inactivation. Disappearance of enzymatic activity was exponential with time and the inactivation was proportional to pyridoxal-5'-phosphate concentration below 25 mM.

Pyridoxal-5'-phosphate (PLP) is a prosthetic group of several enzymes and has been shown to be bound to the epsilon-amino group of lysine residues (1). It has also been shown to react with lysine residues at the phosphate binding sites of several enzymes (2,3,4). This reaction resulted in inactivation of the enzymes, but the inactivation could be prevented by substrate. Horecker and associates (5) have used the reaction with PLP to identify and distinguish between the two phosphate binding sites of rabbit muscle aldolase.

Although microsomal glucose-6-phosphatase (EC 3.1.3.9) has not yet been purified, Feldman and Butler (6) have reported that during the course of the reaction the phosphate group of substrate binds covalently to a histidine at the active site of the enzyme protein. Kinetic studies (7,8) also have suggested that the phosphate group of glucose-6-phosphate and other substrates may be intimately involved in the formation of the enzyme-substrate complex. Therefore, it seemed of interest to study the effect of PLP on the activity of microsomal glucose-6-phosphatase. An initial report of these studies is presented here.

Methods and Materials

Microsomes: A 10% homogenate of liver from 24 hours fasted rats was

prepared in 0.25 M sucrose. Microsomes were prepared from the homogenate by the conventional centrifugation procedure (9). The microsomal residue was washed twice with 0.25 M sucrose and recovered by centrifugation at 105,000 x g. The residue was resuspended in 0.25 M sucrose solution equivalent to one-fourth that of the original homogenate and lyophilized. Lyophilized microsomes were resuspended in ice-cold buffer or water just before use. We have found that glucose-6-phosphatase activity of the lyophilized preparation was quite stable when stored at -15°.

Inactivation by PLP: Lyophilized microsomes were resuspended in 0.05 M Tris-buffer, pH 7.0, and incubated with PLP in the same buffer at 30°. To stop the reaction, an aliquot of the incubation mixture, at desired time intervals, was added into a solution containing L-lysine in 50-fold molar excess over PLP. The mixture was centrifuged at 105,000 x g for 30 minutes and the residue was washed 4 times with the Tris-buffer and separated by centrifugation. The residue was finally suspended in 0.25 M sucrose and used for glucose-6-phosphatase and protein assays (10). The control incubation mixture consisted of PLP and 50-fold molar excess L-lysine to which microsomes were added last.

Glucose-6-phosphatase Activity: Enzymatic activity was measured in 0.1 M acetate buffer, pH 6.1, at 30°. Reaction was started by adding an aliquot of the enzyme suspension to glucose-6-phosphate (0.04 M) and buffer mixture maintained at 30°. Reaction was terminated at the end of 10 minutes by adding an equal volume of 16% trichloracetic acid. The enzyme was added after trichloracetic acid in the control. The mixture was centrifuged and an aliquot of the supernatant was used for phosphate determination (11). Enzyme activity was expressed as μmoles of inorganic phosphate formed per minute per mg protein. All chemicals were obtained from Sigma Chemical Company (St. Louis).

Results and Discussion

When microsomes (3.5 mg protein/ml) were incubated at 30° with 25 mM

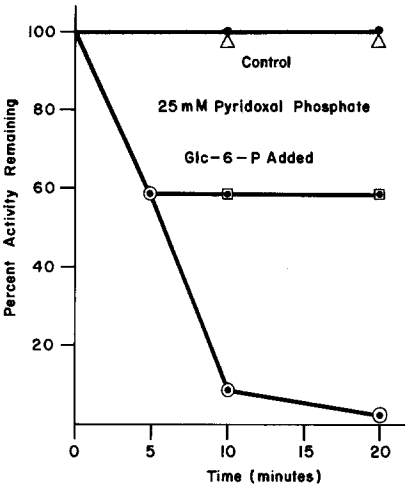

Fig. 1. Effect of PLP (25 mM) on microsomal glucose-6-phosphatase activity (3.5 mg of protein/ml). Reaction mixture was incubated at 30° in 0.05 M Tris-buffer, pH 7.0. ●, control, microsomes were added to a mixture of PLP and excess lysine; Δ, glucose-6-phosphate (30 mM) was added to a mixture of PLP and microsomes at '0' time; ⊙, microsomes and PLP; ⊡, glucose-6-phosphate (30 mM) was added to a mixture of PLP and microsomes after 5 minutes of incubation. See text for more details.

PLP inactivation of glucose-6-phosphatase was almost complete in 10-15 minutes and a plot of the percent remaining activity with time is shown in Figure 1. Inactivation of glucose-6-phosphatase activity by PLP was completely prevented by the inclusion of 30 mM glucose-6-phosphatase in the incubation mixture. Substrate protection was more clearly shown when addition of glucose-6-phosphate to a preincubated mixture protected the enzyme from further inactivation. These results indicate that inactivation by PLP may be due to reaction at the substrate binding site of the enzyme protein. We have observed similar inactivation by PLP of glucose-6-phosphatase activity of human liver microsomes.

Figure 2 shows inactivation of glucose-6-phosphatase activity of rat liver microsomes (3.5 mg protein/ml) at different concentrations of PLP. The percent remaining activity was exponential with time, and inactivation was essentially complete after 20 minutes of incubation at 30°. A semilog plot of these data yielded a straight line (not shown) for each concentra-

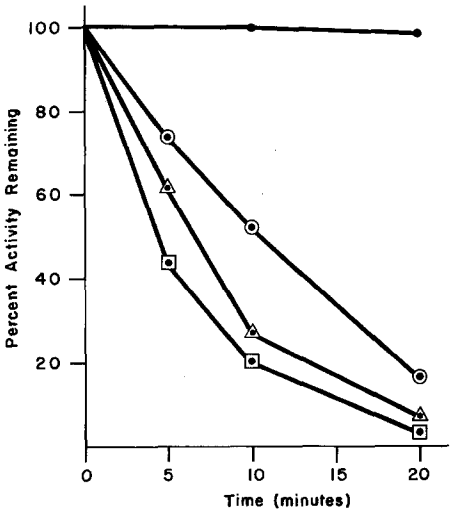

Fig. 2. Effect of PLP concentration on the rate of inactivation of microsomal glucose-6-phosphatase activity. Microsomes were incubated at 30° at different concentrations of PLP in 0.05 M Tris-buffer, pH 7.0. o, control (see text); ⊙, 10 mM PLP; △, 15 mM PLP; ⊡, 20 mM PLP.

tion of PLP except for the highest (25 mM) (Figure 1). This difference at 25 mM PLP was probably due to a higher rate of reaction at sites other than the substrate binding site of the enzyme protein. From slopes of the semilog plots values for pseudo first order rate constants [-dA/dt = k (PLP); A = enzyme activity, (PLP) = molar concentration of PLP, and k = pseudo first order rate constant for PLP] could be obtained.

Although absorption maxima in the 410-430 mu region are typical of Schiff-base formation (12) after reaction of PLP with proteins, identification of the reaction site in microsomal proteins is complicated by the presence of cytochromes which also absorb in this region. However, studies using differential spectrophotometry (to be published) have revealed that at low concentration of PLP the reaction results in the formation of Schiff-bases probably involving epsilon-amino group of lysines at the substrate binding site of the enzyme protein. At higher PLP concentrations or longer reaction times, reaction seems to occur at amino as well as sulfhydryl groups The pH optimum for glucose-6-phosphatase activity is 6.1 at which glucose-

6-phosphate would exist as a dianion (13). Therefore, positively charged amino groups may provide a complimentary structure to the binding of dianionic glucose-6-phosphate.

Acknowledgements. This research was supported by the Meadowbrook Medical Education and Research Foundation, Inc.

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