GLUCOSE-6-PHOSPHATASE AND PYROPHOSPHATASE ACTIVITIES OF HOMOGENATES OF LIVERS FROM PATIENTS WITH GLYCOGEN STORAGE DISEASE*

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It has been reported by Nordlie and Arion (1964) and by Stetten (1964) that three different catalytic activities in a microsomal fraction of liver,

(1) glucose-6-P + H_2O → glucose + P_i

(2) PP_i + H_2O → 2 P_i

(3) PP_i + glucose → glucose-6-P + P_i

may, in fact, reside in the same enzyme. The evidence was based on the occurrence of these activities mainly in liver and kidney, on their distribution in subcellular fractions, on the constancy of the ratios of these activities during various fractionation and inactivation procedures, and on kinetic measurements. Both groups of authors showed that PP_i was a competitive inhibitor of reaction (1) and glucose-6-P of reaction (2), pointing to a common binding site. Glucose, according to Stetten and Taft (1964), was a non-competitive inhibitor of reaction (2), such that under optimal conditions 80-90% of the total PP_i which disappeared was utilized in reaction (3).

The fact that there is a genetically determined deficiency of glucose-6-phosphatase activity in the liver and kidney of patients with Type I glycogen storage disease (Cori and Cori, 1952), offered another approach to the problem presented above.

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Materials and Methods. Liver samples obtained by open biopsy were frozen on dry ice in the operating room, packed in dry ice and stored at -80° C under conditions which prevented dehydration of the tissues. The Type II glycogenosis samples were obtained at autopsy within two hours of death. In order to accumulate a meaningful series, it was necessary to utilize tissues which had been stored from 1 month to 1 year. All had been previously assayed for glucose-6-phosphatase activity within a few days of their receipt as follows: 0.01 M glucose-6-P, 0.03 M citrate, pH 6.7, homogenate equivalent to 5-20 mg of liver, and incubation at 30° for 30-60 minutes.

In the present experiments, homogenates were prepared by grinding 100-150 mg of frozen liver with 9 volumes of cold distilled water in chilled glass homogenizers. The assay conditions were similar to those used by Stetten, except that Triton X - 100 and EDTA were omitted. Glucose-6-phosphatase activity was measured at pH 5.4, 6.1 and 6.9 in the presence of 0.08 M substrate and 0.1 M buffer (acetate at pH 5.4, citrate at the other two pH's). Homogenate equivalent to 10 mg of liver was added to each reaction mixture (final volume 0.25 ml) and incubated at 30° for 20 minutes.

Pyrophosphatase activity was measured at pH 5.4 with 0.08 M substrate, 0.1 M acetate buffer, homogenate equivalent to 10 mg of liver, and an incubation period of 20 minutes at 30°. The hydrolysis of pyrophosphate was also measured at pH 7 in the presence and absence of $MgCl_2$ and the results recorded for the Mg^{++} stimulated pyrophosphatase. The hydrolysis of 0.05 M β-glycerophosphate in 0.05 M acetate buffer at pH 5 was taken as a measure of acid phosphatase activity (Gianetto et al. 1955).

In certain experiments the assays were repeated after aliquots of the homogenates had been adjusted to pH 5 and incubated at 37° for 30 minutes in the absence of substrate (Beaufay et al. 1954). All reactions were stopped by the addition of 1.0 ml of cold 5% tri-

chloroacetic acid, the tubes chilled and centrifuged, and aliquots removed for determination of inorganic phosphate by the Fiske-Subbarow method (1925). Suitable controls for all substrates were incubated at 30° in the absence of homogenate, then treated with trichloroacetic acid, and the values for inorganic P subtracted from the experimental ones which were also corrected for the trace of P_i (separately determined) found in the tissue homogenates. All activities are expressed in terms of μmoles P_i formed per minute per gram of liver.

Results and Discussion. Evidence that the enzyme activities were well preserved in the stored liver samples came from a repetition of the assays for glucose-6-phosphatase which showed in all cases good agreement with the values obtained previously. Since only frozen livers were available, it was not possible to isolate a well separated microsomal fraction by centrifugation or to establish the true ratios of enzymatic activities. In homogenates the assay for glucose-6-phosphatase at pH 5.4 and 6.1 is interfered with to some extent by the acid phosphatase, especially at high substrate concentration. This interference will be greatest in livers with very low glucose-6-phosphatase activity such as those from cases of Type I glycogen storage disease. There are 5 such cases reported in Table I. In only 2 of these was there a minimal hydrolysis of glucose-6-P at neutral pH, whereas 4 of these showed some hydrolysis at the more acid pH. Similarly, after procedures designed to destroy glucose-6-phosphatase, e.g. incubation at pH 5 at 37°, more apparent activity remained at the acid pH than at pH 6.9 (cf. Table I).

The cases of Type I glycogenosis exhibited a markedly diminished ability to hydrolyze inorganic pyrophosphate at pH 5.4 when compared with other types of glycogenosis or with livers without glycogen storage. On the other hand, the neutral, Mg^{++}-dependent pyrophosphatase and the acid phosphatase were not markedly diminished in Type I

TABLE I

ENZYMATIC ACTIVITIES IN HUMAN LIVER HOMOGENATES

(μmoles P_i formed/minute/gram liver)

Glycogenosis Type*	Glucose-6-phosphatase			Pyrophosphatase		Acid Phosphatase
	pH 5.4	pH 6.1	pH 6.9	pH 5.4	pH 7 Mg^{++}	pH 5
I A.P.	0	0	0	0.27		1.70
I A.H.	0.55	0.55	0		3.84	1.36
I A.R.	1.20	0.82	0	1.10	3.28	1.89
I D.M.	0.33	0.55	0.48	0.93	1.31	1.23
I K.B.	0.55	0.77	0.55	0.88	3.61	1.16
II B.P.	6.60	7.80	8.40	13.20	5.05	2.92
After pH 5	[1.83]+	[1.43]	[0.61]	[2.18]	[0.07]	[3.24]
II R.C.	5.82	6.48	5.93	9.00	4.93	5.36
After pH 5	[3.00]	[2.11]	[0.25]	[2.24]		[4.10]
II T. O'D.	3.72	3.78	3.34	6.10	4.12	3.64
After pH 5	[1.66]	[1.26]	[0.46]	[2.19]	[0.86]	[4.21]
II S.M.	4.82	4.77	3.94	8.80	11.10	4.15
III S.G.	1.97	2.58	3.51	3.94	4.95	1.69
VI J.O'B.	2.68	2.25	2.46	2.96	5.01	1.47
—** J.C.	5.50	5.00	4.66	11.20	4.34	
— P.G.	6.20		5.87	9.93	5.82	2.36
— E.F.	4.32	5.04	5.60	9.40	6.59	2.66
— C.E.	2.80	3.28	3.07	7.02	5.60	2.19
After pH 5	[1.24]	[0.88]	[0.15]	[2.04]		[1.67]

*Tissue samples were received from the following physicians: R. Cardiff, Portland, Oregon; G. Donnell, Los Angeles, Calif.; G. Fine, Detroit, Mich.; D. Goldring, St. Louis, Mo.; J. Heersma, Marshfield, Wis.; R. Janeway, Winston-Salem, N.C.; T. Starzl, Denver, Colo.; R. Ulstrom, Minneapolis, Minn.; H. Wohltmann, St. Louis, Mo.; H. Zellweger, Iowa City, Iowa.

+Values in [] obtained after incubation at pH 5 at 37° for 30 min.

**Normal glycogen content in the liver.

glycogenosis. Homogenates which had normal glucose-6-phosphatase and acid pyrophosphatase activity showed a marked decrease in both

of these activities after treatment of the homogenate at pH 5. The Mg^{++}-dependent pyrophosphatase activity was also destroyed by this treatment, whereas the lysosomal acid phosphatase was resistant to such pretreatment at pH 5.

A classification of different types of glycogenosis according to enzymatic defects has been given (Illingworth 1961). Baudhuin et al. (1964) reported a single case of Type II glycogenosis in which the acid phosphatase was increased. In the present series of 4 cases the average acid phosphatase activity was 4.02 μmoles per gram per minute as compared to an average of 1.77 μmoles for all other values reported in Table I.

Based on the one gene-one enzyme hypothesis, the observations with liver tissue from Type I glycogen storage disease lend further support to the idea that the microsomal glucose-6-phosphatase and pyrophosphatase activities are catalyzed by the same enzyme.

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