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The Hydrolysis of Glucose 1-Phosphate by Rat-Liver Extracts

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The existence in rat liver of a phosphomonoesterase specific for the hydrolysis of glucose 6-phosphate is now well established, after the isolation of a partially purified preparation of this enzyme by Swanson (1950). It had been previously suggested by Broh-Kahn & Mirsky (1948) that no specific glucose 1-phosphatase activity exists in liver and that glucose 1-phosphate is converted into glucose 6-phosphate before hydrolysis. Faulkner (1955), on the other hand, has demonstrated the presence in silkworm blood of a phosphatase specific for glucose 1-phosphate. The work of Broh-Kahn & Mirsky (1948) did not entirely eliminate the possibility of hydrolysis of glucose 6-phosphate by other routes, and the present work was undertaken to examine the hypothesis of these workers. A preliminary account of this work has already appeared (Goodlad & Mills, 1956).

EXPERIMENTAL

Substrates. Glucose 1-phosphate and glucose 6-phosphate were prepared as previously described (Goodlad & Mills, 1957).

Phosphatase activity. This was assayed by the general method described by Goodlad & Mills (1957).

Phosphoglucomutase activity. This was measured by a method based on that of Najjar (1948): 0.1 ml. of 0.05 M-glucose 1-phosphate (potassium salt, in solution containing 0.1 M-cysteine), 0.5 ml. of buffer solution, 0.1 ml. of 0.01 M-MgSO₄ and 0.3 ml. of enzyme solution, all preheated to 38°, were incubated together for 15 min. at 38°. The reaction was stopped by the addition of 1 ml. of 5 N-H₂SO₄ followed by 3 ml. of water, and precipitated protein was removed by

centrifuging. A portion (0.5 ml.) of the supernatant was heated at 100° for 10 min. and the total inorganic phosphate was estimated by the method of Allen (1940). The initial acid-labile phosphorus was determined in the same manner except that the substrate was added after the H₂SO₄. The decrease in acid-labile phosphorus (glucose 1-phosphate) is equivalent to the amount of acid-stable glucose 6-phosphate formed.

One unit of phosphoglucomutase activity $\equiv$ 1 μ g. of acid-stable phosphorus formed/hr. under the above conditions.

Separation of cytoplasmic particles. This was carried out by the method of Schneider (1948), which involves differential centrifuging in 0.25 M-sucrose solution. Separations were carried out at 4° with an MSE Major refrigerated centrifuge, a force of 600 g for 10 min. being used to separate nuclei and unbroken cells from the original homogenate. Centrifuging the supernatant at 8500–10 000 g for 10 min. separated the mitochondrial fraction (MT), and centrifuging the supernatant and washings from the MT fraction at 18 000 g for 1 hr. separated the microsomal fraction (MS) and the supernatant cell sap (S). Each fraction was frozen in a solid CO₂-acetone mixture and thawed rapidly, this process being repeated twice more to ensure liberation of enzymic activity from the particles (cf. Berthet, Berthet, Appelmans & de Duve, 1951).

RESULTS

Hydrolysis of glucose 1-phosphate (G 1-P) and glucose 6-phosphate (G 6-P) by unfractionated rat-liver homogenates. The hydrolysis of G 1-P by rat-liver homogenates differs in a number of respects from the hydrolysis of G 6-P. The pH optimum for the hydrolysis of G 1-P was found to lie between

pH 7.2 and 7.5, whereas that for G 6-P was pH 6.5 (Fig. 1). In addition, the speed of hydrolysis of G 6-P was almost twice that of G 1-P, each being measured at the same pH optimum and at the same substrate concentration (5 mM). Magnesium ions were found to activate the hydrolysis of G 1-P to a considerable degree but had little action on the hydrolysis of G 6-P (Fig. 2). There was considerable inhibition of hydrolysis rate at high substrate concentrations with G 1-P but not with G 6-P (Fig. 3).

Fig. 1. Effect of pH on the hydrolysis of G 1-P and G 6-P by rat-liver homogenates. Substrates were (a) 0.005M-G 1-P and (b) 0.005M-G 6-P; 0.001M-Mg²⁺ ions were present in (a). Veronal-acetate buffers were used; temp. 38°. Hydrolysis was followed by rate of liberation of inorganic P. One unit of activity = 1 μ g. of P liberated/hr.

Fig. 2. Effect of Mg²⁺ ions on the hydrolysis of (a) 0.005M-G 6-P and (b) 0.005M-G 1-P by rat-liver homogenates. $\times$, Mg²⁺ ions present; $\bullet$, Mg²⁺ ions absent. Veronal-acetate buffers were used; temp. 38°.

Some properties of rat-liver phosphoglucumutase. The enzyme source in these experiments was the cell sap (S). The preparation used was shown to be free from glucose 6-phosphatase activity (Table 1, liver II), and phosphoglucumutase activity has already been shown to be located entirely in the cell sap by Hers, Berthet, Berthet & de Duve (1951).

The pH optimum for the rat-liver phosphoglucumutase activity was found to be 7.2 (Fig. 4), and Mg²⁺ ions were found to be necessary for activity. Activation by Mg²⁺ ions was found to increase sharply up to a maximum at a concentration of 0.5 mM; thereafter, increasing Mg²⁺ ion concentration caused a fall in activity (Fig. 5). High concentrations of substrate were found to have an inhibitory effect, optimum activity occurring at a concentration of 5 mM-G 1-P (Fig. 6).

The present work indicates that rat-liver phosphoglucumutase has a pH optimum and a Mg²⁺ ion requirement similar to those of preparations of this enzyme from other sources (Najjar, 1948; Cardini, 1951; Sable & Calkins, 1953). In addition, it has been found that liver phosphoglucumutase does not lose significant activity on heating at 63° for 3 min. and that it is precipitated by (NH₄)₂SO₄ at between 40 and 50 % saturation.

Hydrolysis of G 1-P and G 6-P by cytoplasmic fractions of rat liver. In addition to showing that the phosphoglucumutase activity of liver occurs in the cell sap, Hers *et al.* (1951) showed that the glucose 6-phosphatase activity occurs principally in the microsomal fraction.

Since the postulated mechanism for the hydrolysis of G 1-P involves a preliminary conversion into G 6-P, it is necessary to separate the two enzymic activities involved in this mechanism in order that any possible secondary mechanism for

Fig. 3. Effect of substrate concentration on the hydrolysis of G 1-P and G 6-P by rat-liver homogenates at 38°. $\bullet$, G 6-P; $\times$, G 1-P. 0.001M-Mg²⁺ ions were present with G 1-P. Assays were at pH 6.5 for G 6-P and at pH 7.2 for G 1-P.

the hydrolysis of G 1-P may become apparent. Rat-liver cytoplasm was therefore fractionated in 0.25M-sucrose and the fractions were assayed singly, and in various combinations, for activity in hydrolysing G 1-P and G 6-P. The results of such an

Fig. 4. Effect of pH on the phosphoglucumutase activity of rat-liver cell-sap fraction. 0.005M-G 1-P, 0.001M-Mg²⁺ ions and 0.001M-cysteine in veronal-acetate buffers at 38°.

Fig. 5. Effect of Mg²⁺ ion concentration on the phosphoglucumutase activity of rat-liver cell-sap fraction. 0.005M-G 1-P and 0.001M-cysteine at pH 7.7 and 38°.

Fig. 6. Effect of substrate concentration on the phosphoglucumutase activity of rat-liver cell-sap fraction. 0.001M-Mg²⁺ ions and 0.001M-cysteine were present. Assays were carried out at pH 7.7 and 38°.

experiment are presented in Table 1. The distribution of glucose 6-phosphatase activity was found to be similar to that reported by Hers *et al.* (1951), that is, mainly in the microsomal fraction. Combination of the various fractions did not increase the glucose 6-phosphatase activity more than was expected from the individual activities.

The hydrolysis of G 1-P by each of the individual fractions was very small, but on combination of the microsomal and cell-sap fractions a large proportion (85 %) of the original activity was recovered, whereas 40 % of the original activity was present in a combination of the mitochondrial and cell-sap fractions. No significant increases in activity were observed when either the microsomal or the cell-sap fraction was boiled before addition to the cell-sap or microsomal fraction respectively. This would appear to rule out the possibility of the increased activities on combination of the fractions being due to activators, and it suggests that the total activity is due to enzymic action.

These results do not provide any evidence for the direct breakdown of G 1-P by a glucose 1-phosphatase, and the observed hydrolysis of this compound is most readily explained by the simultaneous activities of phosphoglucumutase and glucose 6-phosphatase.

DISCUSSION

Since the hexose phosphates of liver are the ultimate source of blood glucose, the pathway of glucose 1-phosphate breakdown by liver is of considerable physiological importance.

Fleury, Courtois, Anagnostopoulos & Desjoberg (1950) have claimed that purified preparations of alkaline phosphatase from kidney, liver and bone,

Table 1. *Hydrolysis of glucose 1-phosphate and glucose 6-phosphate by rat-liver cytoplasmic fractions*

Activities in the various fractions are expressed as a percentage of the activity shown by a mixture of mitochondria (MT), microsomes (MS) and cell sap (S), in the proportions normally obtained from liver. Substrate concentration = 5 mM; 0.001M-MgSO₄ was added with G 1-P; pH of assay was 7.2 with G 1-P and 6.5 with G 6-P. Incubation was for 1 hr. at 38°.

Cytoplasmic fraction	Liver I Substrate G 6-P	Liver II Substrate	
		G 6-P	G 1-P
MT + MS + S	100	100	100
MT	14	23	6
MS	80	78	8
S	14	0	4
MT + MS	93	—	4
MS + S	82	—	85
MT + S	28	—	40
MS + S*	—	—	17
MS* + S	—	—	9

* Fraction boiled before adding to reaction mixture.

and also purified human prostate phosphatase, can hydrolyse G 1-P more or less rapidly. On the other hand, Swanson (1950) found that her purified preparation of liver glucose 6-phosphatase had very little activity towards G 1-P.

In the present work, the hydrolysis of G 1-P by liver extracts was most active at pH 7.2–7.5, with a rapid decline in activity on either side of the optimum, although the shape of the pH-activity curve did not completely preclude the hydrolysis of G 1-P by non-specific alkaline phosphatases at higher pH values.

The characteristics of the hydrolysis of G 1-P by unfractionated extracts of liver differed from those of glucose 6-phosphatase but closely resembled the characteristics of liver phosphoglucosomutase. These findings lend strong support to the views of Broh-Kahn & Mirsky (1948) that G 1-P undergoes a preliminary conversion into G 6-P before hydrolysis and that this conversion into G 6-P is the rate-controlling step in the over-all reaction. This view is strengthened by the finding that no fraction of liver cytoplasm hydrolysed G 1-P alone, but that combination of either microsomal or mitochondrial fraction with cell sap caused significant hydrolysis. In these cases the microsomal and, to a lesser degree, the mitochondrial fractions contained glucose 6-phosphatase activity whereas the cell sap did not. The cell sap is, however, a rich source of the enzyme phosphoglucosomutase.

The ability of the nuclear fraction to hydrolyse G 1-P was not studied in the present work, but it has been found by one of us (G.T.M., unpublished observation) that isolated rat-liver nuclei cause no significant breakdown of G 1-P.

SUMMARY

1. The hydrolysis of glucose 1-phosphate (G 1-P) by unfractionated rat-liver homogenate differs from

the hydrolysis of glucose 6-phosphate (G 6-P) in having a higher optimum pH, being much more strongly activated by Mg^{2+} ions and by showing inhibition by high substrate concentrations.

2. Some properties of liver phosphoglucosomutase have been investigated and the enzyme was found to be similar in many respects to that isolated from other tissues.

3. The results of the present work support the view that the main route of G 1-P hydrolysis in liver is through a preliminary conversion into G 6-P, catalysed by phosphoglucosomutase.

4. An examination of the abilities of the separated cytoplasmic fractions to hydrolyse G 1-P and G 6-P supports the above view.

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Studies on Sulphatases

16. A SOLUBLE PREPARATION OF ARYLSULPHATASE C OF RAT-LIVER MICROSOMES*

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The arylsulphatase activity of rat liver can be attributed to at least three distinct enzymes which can be differentiated by their relative substrate specificities, behaviour towards inhibitors and their localization within the liver cell. Two of the aryl-

sulphatases (A and B) have very similar properties and are found mainly in the mitochondria. They are easily obtained in a soluble form by any of the procedures which are known to disrupt mitochondria, and have been termed the 'soluble' arylsulphatases. On the other hand, the remaining enzyme (aryl-sulphatase C), which is located almost exclusively in the microsomes, has been termed 'insoluble' since

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