Inhibition of Glycerol Kinase by α -Glycerophosphate

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Some kinetic properties of glycerol kinase (EC 2.7.1.30) isolated from rat, ox and human liver and from *Candida mycoderma* were reported by Grunnet & Lundquist (1967*a,b*). In contrast with the results of Robinson & Newsholme (1967, 1969*b*) with rat liver glycerol kinase we observed no product inhibition by α -glycerophosphate. Experiments to resolve this discrepancy have now been carried out.

The only apparent difference between the experiments of Robinson & Newsholme (1967, 1969*b*) and our experiments was the assay method used. Robinson & Newsholme (1967, 1969*b*) measured the reaction by a radiochemical assay (Newsholme, Robinson & Taylor, 1967; Robinson & Newsholme, 1969*b*), whereas we used coupled enzymic methods, requiring additions of salts, cofactors and substrates not necessary for the glycerol kinase itself. To study the effect of salts and cofactors on the inhibition of glycerol kinase by α -glycerophosphate I have adopted the radiochemical assay method.

The experiments show that partially purified glycerol kinase from rat liver is inhibited by α -glycerophosphate in the absence of phosphate or sulphate, whereas the presence of either one of these ions makes the enzyme insensitive to α -glycerophosphate inhibition. Glycerol kinase activity in a rat liver homogenate is inhibited by α -glycerophosphate whether phosphate or sulphate is present or not.

Materials and methods. All chemicals were analytical grade. DL-3-Glycerophosphate containing 48% L-3-glycerophosphate was used. Glycerol kinase from rat liver was prepared as described by Grunnet & Lundquist (1967*b*). Coenzymes and phosphoenolpyruvate was purchased from C. F. Boehringer und Soehne G.m.b.H. (Mannheim, Germany). [1,3- ^{14}C]Glycerol (specific radioactivity 6.7 mCi/mmol) was obtained from New England Nuclear Corp. (Boston, Mass., U.S.A.) and the ion-exchange paper used was Whatman DE 81 (W. and R. Balston Ltd., Maidstone, Kent, U.K.). Radioactivity was measured to a minimum of 10000 counts. Glycerol kinase activity was determined spectrophotometrically (Grunnet & Lundquist, 1967*b*) or by using the radiochemical assay method of Newsholme *et al.* (1967).

Results and discussion. In the radiochemical assay proportionality was obtained between radioactivity incorporated into α -glycerophosphate and

time or the amount of enzyme added to the incubation mixture.

The results of experiments designed to examine the inhibition by α -glycerophosphate are shown in Table 1. The values given are the means of at least three experiments. It is seen that α -glycerophosphate inhibits rat liver glycerol kinase in the absence of phosphate or sulphate. Assuming competitive inhibition with respect to glycerol (Robinson & Newsholme, 1969*b*) K_i for L-3-glycerophosphate was calculated to be $3.9 \times 10^{-4}\text{M}$. This is in accordance with the value of $5.8 \times 10^{-4}\text{M}$ obtained by Robinson & Newsholme (1969*b*). Glycerol kinase was protected from α -glycerophosphate inhibition by ammonium sulphate (Table 1), but not by any of the other salts, cofactors and enzymes used in the coupled enzymic assay. Sodium sulphate has the same effect, so the protecting effect should be ascribed to the sulphate ion. Phosphate has, even in concentrations below 0.1 mM, the same effect as sulphate. The protective effect of sulphate or phosphate is apparently due to competition between α -glycerophosphate and the anion.

Assays with the enzymic method previously used showed similar results when the lactate dehydrogenase and pyruvate kinase solutions were dialysed before use.

Glycerol kinase activity measured in an 8% (w/v) rat liver homogenate, prepared in 0.15 M-potassium chloride-1 mM-EDTA, centrifuged for 130000 g-min and passed through a Sephadex G-25 column before use, was inhibited by α -glycerophosphate to the same extent as the partially purified enzyme. Inorganic phosphate or sulphate did not, however, protect the enzyme in the homogenate against inhibition by α -glycerophosphate (Table 1).

In experiments with a mixture of a rat liver homogenate and the purified enzyme reaction rates were obtained that were the sum of the rate measured with the homogenate enzyme and that measured with the purified enzyme, whether phosphate and/or α -glycerophosphate were present or not (results not shown in Table 1). This result excludes the possibility that the protecting effect of phosphate or sulphate is counteracted by some factor in the liver homogenate.

The lack of inhibition of glycerol kinase by α -glycerophosphate when inorganic phosphate or sulphate is present thus appears to be an artifact

Table 1. *Determination of glycerol kinase activity by the method of Newsholme et al. (1967)*

The incubation mixture contained (final concentrations) 90 mM-tris, 1.8 mM-ATP, 1.8 mM-MgCl₂, 0.09 mM-[1,3-¹⁴C₂]glycerol, partially purified glycerol kinase from rat liver (Grunnet & Lundquist, 1967b) or a rat liver homogenate that had been passed through a Sephadex G-25 column before use, plus the compounds listed in the table, adjusted to pH 7.5 and in a total volume of 0.11 ml.

	Concn. of L-3-glycero- phosphate (mM)	Concn. of sodium phosphate (mM)	Further addition	Glycerol kinase activity (c.p.m. incorporated into product/3 min per 10 µl)	Activity (%)
Purified rat liver glycerol kinase	0	0	0	3550	100
	0	0.4	0	3550	100
	0	0.8	0	3550	100
	0	21	0	3600	102
	1.1	0	0	2460	69
	1.1	0.08	0	3740	105
	1.1	0.4	0	3200	90
	1.1	0.8	0	3720	105
	1.1	21	0	3550	100
	1.1	0	(NH ₄) ₂ SO ₄ (0.08 mM)	2420	68
	1.1	0	(NH ₄) ₂ SO ₄ (0.8 mM)	3460	98
	1.1	0	(NH ₄) ₂ SO ₄ (30 mM)	3575	101
	1.1	0	Salts or cofactors*	2430	68
	4.5	0	0	1380	39
	4.5	0.8	0	3000	85
Rat liver	0	0	0	5300	100
homogenate	0	10	0	5450	103
	1.1	0	0	3900	74
	1.1	10	0	3800	72

* 120 mM-KCl, 0.2 mM-NADH, 0.7 mM-phosphoenolpyruvate or 1.5 units of pyruvate kinase plus 3 units of lactate dehydrogenase (both dialysed).

created during the preparation procedure. Product inhibition of the enzyme by α -glycerophosphate should therefore be taken into account when the metabolism of glycerol is being considered.

Although some factor other than the known kinetic properties of the glycerol kinase appears to regulate the rate of glycerol uptake by liver cells (Robinson & Newsholme, 1969a), one may expect a rapid change of the intracellular concentration of glycerol when the extracellular concentration of this metabolite is changed (Hayashi & Lin, 1965). When a high extracellular concentration of glycerol is present, as in the case of inhibition of glycerol utilization by ethanol (Lundquist, Tygstrup, Winkler & Jensen, 1965; Thieden, 1969), one would expect only about 1% inhibition by the steady-state concentration of α -glycerophosphate obtained (Zakim, 1965; Thieden, 1969).

AMP, which accumulates in the liver after administration of ethanol (Thieden, 1969) in concentrations that would correspond to about 20% inhibition of glycerol kinase under the conditions used (Grunnet & Lundquist, 1967b), may be a more likely cause of the observed inhibition (Lund-

quist *et al.* 1965; Thieden, 1969) of glycerol metabolism.

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- Grunnet, N. & Lundquist, F. (1967a). *Abstr. FEBS 4th Meet., Oslo*, no. 258.
 Grunnet, N. & Lundquist, F. (1967b). *Eur. J. Biochem.* **3**, 78.
 Hayashi, S. & Lin, E. C. C. (1965). *Biochim. biophys. Acta*, **94**, 479.
 Lundquist, F., Tygstrup, N., Winkler, K. & Jensen, K. B. (1965). *Science, N.Y.*, **150**, 616.
 Newsholme, E. A., Robinson, J. & Taylor, K. (1967). *Biochim. biophys. Acta*, **132**, 338.
 Robinson, J. & Newsholme, E. A. (1967). *Abstr. FEBS 4th Meet., Oslo*, no. 64.
 Robinson, J. & Newsholme, E. A. (1969a). *Biochem. J.* **112**, 449.
 Robinson, J. & Newsholme, E. A. (1969b). *Biochem. J.* **112**, 455.
 Thieden, H. I. D. (1969). *Acta chem. scand.* **23**, 237.
 Zakim, D. (1965). *Archs Biochem. Biophys.* **111**, 253.