The substrate specificity of liver glucose-6-phosphatase

The recent studies of BEAUFAYS AND DE DUVE[1,2] have shown that liver glucose-6-phosphatase acts on primary phosphoric esters of a wide variety of compounds such as phenylphosphate, ethyl phosphate, and α-glycerophosphate. In addition, they showed that the hexose ester, fructose-6-phosphate, is a substrate for the enzyme. Our interest in this enzyme arose from the availability of a number of phosphoric esters of sugars and sugar derivatives ranging from 5 to 7 carbon atoms in chain length[3] and the possibility that some of these might prove useful as competitive inhibitors of the action of the enzyme on glucose-6-phosphate.

In Table I are given the approximate values of the Michaelis constants (K_m) of some of the esters tested and the relative rates of hydrolysis at the stated substrate concentrations. Extrapolation of the relative rates to infinite substrate concentrations would largely affect the values only for galactose-6-P and L-sorbose-1-P. The rates found with the various substrates are not widely different except that ribose-5-P and L-sorbose-1-P appear to be hydrolyzed somewhat less rapidly than the other substrates. The K_m values, on the other hand, appear to vary over a 25-fold range of concentrations. Great emphasis, however, should not be placed on the absolute numerical values. In most instances the values given could be confirmed by experiments performed with different enzyme preparations, but an occasional experiment yielded data indicative of K_m values two or three-fold higher than these.

TABLE I

APPROXIMATE MICHAELIS CONSTANTS AND RELATIVE RATES FOR LIVER GLUCOSE-6-PHOSPHATASE WITH VARIOUS PHOSPHATE ESTERS

Ester	*Km Moles/liter*	*Relative rate*	*Substrate concentration Moles/liter*
Glucose-6-P	0.002	1	0.025
2-Deoxyglucose-6-P	0.013	*	—
Galactose-6-P	0.05	0.4	0.033
1,5-Sorbitan-6-P	0.01	0.75	0.025
Allose-6-P	0.007	0.9	0.033
Glucosamine-6-P	0.01	*	—
Ribose-5-P	0.005	0.3	0.033
Glucoheptulose-7-P	0.018	*	—
Mannose-6-P	0.02	0.5	0.07
L-Sorbose-1-P	0.008	0.15	0.008

* Not determined.

The enzyme preparation was the microsome fraction of rat liver (Sprague-Dawley, fed *ad lib.*) isolated from 0.25 *M* sucrose homogenates[7]. The mitochondria were removed by centrifugation at 9000 × *g* for 10 minutes. One-tenth volume of 1.5 *M* KCl was then added and the microsome fraction was collected by centrifugation at 13,000 × *g* for 45 minutes. The microsome fraction was washed with 0.2 *M* histidine, pH 6.7 and suspended for use in the same buffer. These preparations exhibited essentially no activity with glucose-1-phosphate, and a trace of activity with β-glycerophosphate as substrate. All incubations were made at 30° for periods of time ranging as necessary from 5 to 60 minutes. All substrates were neutralized to pH 6.7 and the incubated mixture contained 0.04 *M* histidine, pH 6.7. Maximal activity of the enzyme is at pH 6.0–6.3[4,5]. A small influence of pH in the range of pH 6.3–7.1 on the K_m values could be detected. Assay of activity was made by measurement of liberated inorganic phosphate[8].

Glucose-6-phosphatase is a particulate enzyme[4,5]. The value for the Michaelis constant of galactose-6-phosphate obtained with a preparation treated with a detergent (Triton X-100) known to cause partial dissolution of particulates of this kind[6] showed no difference from that obtained with the untreated preparation although some destruction of enzyme occurred and the maximal rate was lower in the presence of detergent.

The data of Table II illustrate the inhibition of dephosphorylation of one substrate by the presence of another and provides some assurance that the observed hydrolyses are catalyzed by a single enzyme. The extent of inhibition is roughly in accord with the K_m values given in Table I.

The report[1] that fructose-6-phosphate is dephosphorylated by liver glucose-6-phosphatase has been confirmed by following the appearance of free fructose[10] during incubation of this ester with the enzyme. Owing to the presence of traces of phosphoglucose isomerase in the preparations, however, it was impossible to determine the Michaelis constant and relative rate with fructose-6-phosphate as substrate. Typical data obtained were the linear formation during 120 minutes of incubation of 3.0 μmoles of total reducing sugar and 1.6 μmoles of free fructose. The rate of fructose formation was only about 10% of that which would have been obtained with glucose-6-phosphate

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TABLE II

INHIBITION OF LIVER GLUCOSE-6-PHOSPHATASE BY 1,5-SORBITAN-6-P

Enzyme action was followed by the liberation of free sugar[9]. 1,5-Sorbitan is non-reducing.

Substrate	*Substrate concentration moles/liter*	*1,5-Sorbitan-6-P concentration moles/liter*	*Per cent inhibition*
Glucose-6-P	0.0143	0.0214	11
Galactose-6-P	0.0143	0.0214	45
Allose-6-P	0.0143	0.0214	30
L-Sorbose-1-P	0.00625	0.0188	50

and may reflect competitive inhibition by the glucose ester formed through isomerase action. Neither fructose-1,6-di-phosphate nor 6-phosphogluconate were attacked at appreciable rates.

As judged from the fact that the lowest K_m value is exhibited with glucose-6-phosphate as substrate, this ester appears to be the primary substrate and when present in a mixture of esters, as may occur under physiological conditions, it may be presumed to be preferentially dephosphorylated.

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[1] C. DE DUVE, *Ciba Foundation Colloquia on Endocrinology*, 6 (1953) 22.
[2] H. BEAUFAYS AND C. DE DUVE, *Bull. soc. chim. biol.*, (in press).
[3] R. K. CRANE AND A. SOLS, *J. Biol. Chem.*, 210 (1954) 597.
[4] M. SWANSON, *J. Biol. Chem.*, 184 (1950) 647.
[5] H. G. HERS, J. BERTHET, L. BERTHET AND C. DE DUVE, *Bull. soc. chim. biol.*, 33 (1951) 21.
[6] R. K. CRANE AND A. SOLS, in *Methods in Enzymology*, S. P. COLOWICK AND N. O. KAPLAN, Editors, Academic Press, Vol. I (1955).
[7] W. C. SCHNEIDER AND G. H. HOGEBOOM, *J. Biol. Chem.*, 183 (1950) 123.
[8] C. H. FISKE AND Y. SUBBAROW, *J. Biol. Chem.*, 66 (1925) 375.
[9] M. SOMOGYI, *J. Biol. Chem.*, 160 (1945) 69; 195 (1952) 19.
[10] J. H. ROE, J. H. EPSTEIN AND N. P. GOLDSTEIN, *J. Biol. Chem.*, 178 (1949) 839.

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Lupin seed proteins

II. A PHYSICO-CHEMICAL STUDY OF THE PROTEINS FROM YELLOW LUPIN SEED (*LUPINUS LUTEUS*)

In part I[1] the physico-chemical properties of the globulins from blue lupin seed (*Lupinus angustifolius*) were described. The proteins from yellow lupin seed (*Lupinus luteus*) were examined similarly. The present communication describes the results of this examination.

The proteins of defatted and decorticated yellow lupin seed meal were extracted with 10% sodium chloride and then precipitated by adding ammonium sulphate to 85% saturation. Measurements on a Spinco electrically-driven ultracentrifuge revealed (Fig. 1(a)) three major components of sedimentation constants 11.6, 7.4 and 2.0 (designated as the $s_{11.6}$, $s_{7.4}$ and $s_{2.0}$ components) for this preparation in buffer of ionic strength (I) = 0.31 and pH = 7.0. This preparation was very yellow in colour and it was therefore considered necessary, for reasons outlined previously[1], to attempt to remove the colouring matter from the meal before the proteins were extracted. Complete removal of these impurities was effected by repeated extraction of the meal with cold water using a Waring blendor.

The proteins of purified lupin seed meal were again extracted with 10% sodium chloride and precipitated by 85% saturation with ammonium sulphate. Fig. 1(b) shows sedimentation diagrams of this preparation in buffer I = 0.31, pH = 7.0. Here again three components ($s_{11.6}$, $s_{7.4}$ and $s_{2.0}$) were observed. When Fig. 1(b) is compared with Fig. 1(a) a decrease in concentration of the $s_{2.0}$ component is noticed. Hence some of the $s_{2.0}$ component was removed by the water extraction used for the purification of the meal. The proteins extracted in the water wash were precipitated