A specific fructokinase in peas*

A new type of fructokinase has been identified in green pea seeds.

Pea seed homogenates were found to have in addition to a particulate hexokinase (which can phosphorylate glucose, fructose, and 2-deoxyglucose, and which is competitively inhibited by N-acetylglucosamine) a soluble enzyme which appeared to phosphorylate fructose but not glucose or L-sorbose. Since these "fructokinases" readily phosphorylate L-sorbose[1,2], purification of this enzyme was undertaken with the purpose of characterization of its substrate specifity.

Homogenates were fractionated by centrifugation, salting out with ammonium sulfate, and adsorption of extraneous proteins with charcoal. The purified preparations were essentially free of hexokinase and contained little phosphofructokinase; ATPase activity was slight in the conditions used; phosphoglucose isomerase activity was over five times greater than that of the fructokinase. The latter is protected by metal-binding agents; in addition it is activated by cysteine. Activity and stability are maximal in the pH range 6.5 to 8.0.

The purified preparations were incubated with fructose, ATP-Mg, cysteine, fluoride, phosphate, and tris(hydroxymethyl)-aminomethane, at pH 7.5. Phosphorylation was followed by disappearance of sugar[3]. No fructose disappeared if the ATP was omitted. With excess ATP free fructose disappears completely. The kinetics suggest a high affinity for fructose (the K_m must be smaller than $1 \cdot 10^{-3} M$) and no inhibition by accumulated products.

A number of related sugars have been tested for phosphorylation and for inhibition of fructose phosphorylation. The relatively high concentration of $5 \cdot 10^{-2} M$ was chosen to increase the possibility of detecting low affinity substrates or inhibitors. Phosphorylation was measured by

TABLE I

SUBSTRATE SPECIFICITY OF PEAS FRUCTOKINASE

Purified enzyme (2 units[3]) was incubated (30°) with 1.7 μM of cysteine, 17 μM of NaF, potassium phosphate and Tris, 10 μM ATP, 5 μM $MgSO_4$, at pH 7.5, and sugars* as follows: 16.7 μM when tested as substrates; 3.3 μM of fructose plus 16.7 μM of the sugar tested as inhibitor. Total volume was 0.33 ml. Incubation times were 0, 10, and 30 minutes when fructose was present and 0, 30 and 90 minutes in the other cases. For methods see the text.

	Relative rates at $5 \cdot 10^{-2} M$	*Relative affinities***
Fructose	1.00	1.00
L-Sorbose	< 0.05	< 0.02
Mannoheptulose	< 0.05	0.03
Sedoheptulose***	< 0.20	—
1-Methylfructose	< 0.05	< 0.02
3-Methylfructose	< 0.05	< 0.02
Glucose	0.08§	< 0.02
Mannose	0.06	< 0.02
2-Deoxyglucose	< 0.05	< 0.02
N-Acetylglucosamine	< 0.05	< 0.02
Galactose	< 0.05	< 0.02
Arabinose	< 0.05	0.03
Xylose	< 0.05	< 0.02
Ribose	< 0.05	—
L-Arabinose	< 0.05	—

* Sedoheptulose was prepared by acid hydrolysis of sedoheptulosan monohydrate kindly supplied by Dr. N. K. RICHTMYER; 1-methylfructose and 3-methylfructose were a gift of Dr. T. Z. CSAKY; L-arabinose was purchased from the British Drug Houses, Ltd.; the other sugars were obtained as described in a previous paper[3].

** Calculated from $\frac{K_m F}{K_m X} = \frac{(F)\ (V_F - V_{F+X})}{(X)\ V_{F+X}}$, where F stands for fructose and X for the compound tested as inhibitor. This formula was applied after knowing from the results in the previous column that none of these compounds had any high phosphorylation rate at the concentration used. No attempt has been made to substantiate as truly competitive the inhibition observed in two cases because it was too small.

*** By substrate disappearance at $2.5 \cdot 10^{-2} M$ concentration.

§ It has been verified that no isomerization to fructose occurs during incubation of glucose in the above conditions except for the absence of ATP.

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estimation of esterified sugar as previously described[4]; the analyses were carried out by one or more of the methods for ketoses[5], pentoses[6] or carbohydrates (anthrone[7]), as appropiate. The results are summarized in Table I. Out of the fourteen sugars other than fructose tested there was only a slight activity with glucose and mannose. None of the sugars tested seems to have much affinity for the enzyme. These results suggest a substrate specifity unusually high among enzymes phosphorylating hexoses.

The product of the phosphorylation of fructose accumulated as a mixture of phosphoric esters which corresponds to an approximately equilibrated mixture of fructose-6-P and glucose-6-P (Table II). The possibility of fructose-1-P being the primary reaction product can be excluded because of the inability of the system to form aldose out of added fructose-1-P. The primary product appears then to be fructose-6-P.

TABLE II

REACTION PRODUCT

Incubation with fructose as in Table I but without phosphate and on a larger scale. The nucleotide-free filtrate was fractionated with barium acetate and ethanol. The Ba-insoluble fraction was negligible, giving only traces to the ketose test. The results given below correspond to the Ba-alcohol insoluble fraction.

	Standard	*μM*
Ketose[5]	fructose-6-P	2.8
Ketose[5] Ba-Zn soluble	fructose-6-P	0.0
Reducing sugar[8]	fructose-6-P	6.2
Pentose[6]	ribose	0.0*
Phosphate[9] inorganic		0.0
Phosphate[9] acid labile**		0.4
Phosphate[9] total		7.4

* An apparent value of 0.35 μM was found. It can be accounted for by non-pentose reducing sugars. ** Hydrolysed by boiling in N H_2SO_4 for 11 minutes.

Phosphorylation of fructose at C-6 makes the existence of some activity on the corresponding aldoses glucose and mannose more understandable. It must be emphasized that although the rates observed at a substrate concentration of $5 \cdot 10^{-2} M$ are probably less than maximal, the phosphorylation coefficients[3] would be considerably smaller than might appear from these figures.

It appears that among the enzymes phosphorylating fructose the name fructokinase should be restricted to those having a high substrate specificity, as the one here described. For the liver enzyme the appropiate name seems to be ketokinase[1] since it can readily phosphorylate several ketohexoses[1,2] and at least one ketoheptose (mannoheptulose[10]). Those of muscle[11] and intestinal mucosa[4], as well as that of *Schitosoma mansoni*[12] would require further characterization*.

The existence in peas of a fructokinase may help to explain the mechanism of the apparent accumulation of glucose as the sucrose content diminishes in certain stages of peas germination[13].

Laboratorio de Enzimologia, Instituto de Fisiologia, Facultad de Medicina, Madrid (Spain)

ANTONIA MEDINA**
ALBERTO SOLS

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* If an enzyme were found which phosphorylates fructose specifically at C-1, it ought to be given the name fructokinase. Then the C-6 phosphorylating enzyme could be designated as fructokinase.

** From the Instituto de Edafologia y Fisiologia Vegetal, C.S.I.C., Madrid.