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The purification and properties of human liver ketohexokinase

A role for ketohexokinase and fructose-bisphosphate aldolase in the metabolic production of oxalate from xylitol

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Ketohexokinase (EC 2.7.1.3) was purified to homogeneity from human liver, and fructose-bisphosphate aldolase (EC 4.1.2.13) was partially purified from the same source. Ketohexokinase was shown, by column chromatography and polyacrylamide-gel electrophoresis, to be a dimer of M_r 75000. Inhibition studies with *p*-chloromercuribenzoate and *N*-ethylmaleimide indicate that ketohexokinase contains thiol groups, which are required for full activity. With D-xylulose as substrate, ketohexokinase and aldolase can catalyse a reaction sequence which forms glycolaldehyde, a known precursor of oxalate. The distribution of both enzymes in human tissues indicates that this reaction sequence occurs mainly in the liver, to a lesser extent in the kidney, and very little in heart, brain and muscle. The kinetic properties of ketohexokinase show that this enzyme can phosphorylate D-xylulose as readily as D-fructose, except that higher concentrations of D-xylulose are required. The kinetic properties of aldolase show that the enzyme has a higher affinity for D-xylulose 1-phosphate than for D-fructose 1-phosphate. These findings support a role for ketohexokinase and aldolase in the formation of glycolaldehyde. The effect of various metabolites on the activity of the two enzymes was tested to determine the conditions that favour the formation of glycolaldehyde from xylitol. The results indicate that few of these metabolites affect the activity of ketohexokinase, but that aldolase can be inhibited by several phosphorylated compounds. This work suggests that, although the formation of oxalate from xylitol is normally a minor pathway, under certain conditions of increased xylitol metabolism oxalate production can become significant and may result in oxalosis.

There has been renewed interest in the pathways for the metabolism of sugars and sugar alcohols. For example, Williams and co-workers (Williams, 1980; Williams *et al.*, 1984; Bleakley *et al.*, 1984) have stimulated intense debate (Rognstad *et al.*, 1982; Landau & Wood, 1983) with their formulation of a second pentose phosphate pathway. The enzymes for the metabolism of sugar alcohols in human brain have been described (O'Brien & Schofield, 1980; O'Brien *et al.*, 1983). Xylitol is generally considered to be metabolized via the glucuronate–xylulose and pentose phosphate pathways, but its metabolism, both *in vivo* and *in vitro*, has been associated with the production of oxalate and known oxalate precursors (Hannett *et al.*, 1977; Rofe *et al.*, 1977, 1979, 1980; Hauschildt & Brand, 1979).

Only recently has a satisfactory metabolic pathway been proposed to explain oxalate production from xylitol. D-Xylulose, which can be formed from xylitol by the enzyme polyol dehydrogenase (Hollman & Touster, 1957; Arsenis & Touster, 1969; O'Brien *et al.*, 1983), can be phosphorylated to xylulose 1-phosphate by ketohexokinase (ATP: D-fructose 1-phosphotransferase, EC 2.7.1.3) (commonly called 'fructokinase' in the earlier literature), and this phosphate ester can then be cleaved by fructose-bisphosphate aldolase (D-fructose-1,6-

Abbreviation used: SDS, sodium dodecyl sulphate.

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bisphosphate D-glyceraldehyde-3-phosphate-lyase, EC 4.1.2.13) to dihydroxyacetone phosphate and glycolaldehyde. Glycolaldehyde is a known oxalate precursor, and we have shown that ketohexokinase and aldolase, partially purified from human liver, can produce glycolaldehyde from xylulose (James *et al.*, 1982). Independently, it has also been shown that the same reactions can be catalysed by bovine liver ketohexokinase and rat liver aldolase and that xylulose 1-phosphate is produced in isolated hepatocytes that are metabolizing xylulose (Barngrover *et al.*, 1981; Barngrover & Dills, 1983).

The present paper describes the purification of ketohexokinase and aldolase from human liver and examines their properties. In addition, the activity of these enzymes has been studied to determine the conditions under which the metabolism of xylitol will favour the production of the oxalate precursor, glycolaldehyde.

Materials

Human tissues were obtained from the Division of Tissue Pathology in our Institute. The tissues were macroscopically free of disease and taken, within 24h of death, from cadavers which had been stored at 4°C. The tissues were used immediately on receipt.

ATP, ADP, phosphoenolpyruvate, fructose 1-phosphate, fructose 1,6-bisphosphate and suspensions of pyruvate kinase, lactate dehydrogenase, triosephosphate isomerase, glycerol-3-phosphate dehydrogenase, glycerokinase, sorbitol dehydrogenase, aldehyde dehydrogenase and aldolase were purchased from Boehringer Mannheim, Sydney, N.S.W., Australia. Xylulose, glycolaldehyde, SDS, phenylmethanesulphonyl fluoride, dithiothreitol and glycollate oxidase from spinach were from Sigma Chemical Co., St. Louis, MO, U.S.A. *N*-Ethylmaleimide and *p*-chloromercuribenzoate were from Calbiochem–Behring, Sydney, N.S.W., Australia. Sephacryl S-200, DEAE-Sepharose, CM-Sephadex, Chromatofocusing resin, Blue Sepharose and Polybuffer were obtained from Pharmacia (South Seas) Pty. Ltd., Sydney, N.S.W., Australia. Acrylamide, bisacrylamide and *NNN'N'*-tetramethylethylenediamine were from Eastman Kodak Co., Rochester, N.Y., U.S.A. All radiochemicals were obtained from Amersham (Australia) Pty. Ltd., Sydney, N.S.W., Australia. Other chemicals were of the highest purity available. All sugars and sugar alcohols were of the D-form.

Methods

Preparation of D-*xylulose 1-phosphate*

Xylulose 1-phosphate was prepared by a modification of the method of Byrne & Lardy (1954). The eluate from the Amberlite CG-400 (200 mesh) column was assayed for total and inorganic phosphate (Ames, 1966), xylulose 1-phosphate (Barngrover *et al.*, 1981), fructose 1,6-bisphosphate (Michal & Beutler, 1974) and glycolaldehyde (Datta & Racker, 1961). The fractions containing xylulose 1-phosphate were pooled and dried at 45°C under vacuum on a rotary evaporator. The dry residue was washed with water in the rotary evaporator and made up to 4 ml with water, neutralized with KOH and stored at −20°C. The yield of xylulose 1-phosphate was 80% from fructose 1,6-bisphosphate, and the final product contained less than 2% P_i and no detectable triose phosphate.

Enzyme assays

Ketohexokinase activity was measured either radiochemically or spectrophotometrically as described by Adelman *et al.* (1967). Control activities with the spectrophotometric assay were measured by omitting ATP (for polyol dehydrogenase activity), omitting fructose (for ATPase activity) and omitting fructose and ATP (for NADH dehydrogenase activity) as described previously (James *et al.*, 1982). The activity was also measured with 1 mM-fructose or 1 mM-xylulose as the substrate.

Aldolase activity was measured, with fructose 1,6-bisphosphate as the substrate, by using the Calbiochem-Behring (Australia) Aldolase Stat-Pak, which is based on the method of Pinto *et al.* (1969). The activity of aldolase with fructose 1-phosphate or xylulose 1-phosphate was determined by a similar method, except that the triosephosphate isomerase was omitted. Control assays did not contain substrate.

The activity of the coupled reaction sequence involving ketohexokinase and aldolase was examined by measuring the formation of dihydroxyacetone phosphate with glycerol-3-phosphate dehydrogenase and NADH or by measuring the formation of glycolaldehyde with alcohol dehydrogenase and NADH (James *et al.*, 1982).

All enzyme assays were carried out at 37°C.

Homogenates for enzyme assays

Tissues were rinsed with cold 0.9% (w/v) NaCl to remove blood, cut into thin slices and placed in 50mM-triethanolamine/HCl buffer, pH7.5, containing 250mM-sucrose, 5mM-EDTA, 10mM-$MgCl_2$ and 25mM-2-mercaptoethanol. Liver, kidney and brain extracts were prepared with three passes of a loosely fitting Teflon pestle in a glass homogenizer, and heart and skeletal muscle were homogenized for 2 × 15s in a Sorvall Omni-Mixer. Assays were performed on the supernatant fractions which were obtained by immediate centrifugation of the homogenate at 15000*g* for 20min at

4°C. For the measurement of ketohexokinase activity in these crude supernatant fractions, the assay contained, in 20mM-triethanolamine/HCl buffer, pH7.5: 100mM-KCl, 20mM-$MgCl_2$, 15mM-ATP, 0.6mM-NADH, 16mM-NaF (to inhibit ATPase activity), 10units of aldolase/ml, 8units of glycerol-3-phosphate dehydrogenase/ml, 2 units of glycerokinase/ml and 60 units of alcohol dehydrogenase/ml. Depending on which substrate is used, the assay measures the glyceraldehyde or glycolaldehyde formed with alcohol dehydrogenase and NADH. However, because dihydroxyacetone phosphate inhibits aldolase, this is removed by using glycerol-3-phosphate dehydrogenase and glycerokinase. This modified assay system was necessary because, if the ATP depletion was monitored, there are several reactions which would contribute to the apparent ketohexokinase activity, including hexokinase and phosphofructokinase if fructose was the substrate and xylulokinase if xylulose was the substrate.

Protein assays

Protein concentration was determined by the procedure of Lowry *et al.* (1951), except that the protein was first precipitated with 5vol. of 10% (w/v) trichloroacetic acid to prevent interference by sucrose, EDTA or 2-mercaptoethanol. The relative amounts of protein in the column effluents were estimated from the A_{280}.

Purification of human liver ketohexokinase

Human liver ketohexokinase was prepared by a modification of the methods used to prepare the rat (Sanchez *et al.*, 1971*a*) and ox (Raushel & Cleland, 1977) liver enzymes (see Table 1). All purification steps were carried out at 4°C. The Chromatofocusing purification step was performed as described by Sluyterman & Elgersma (1978). The enzyme was then concentrated with an Amicon concentrator and dialysed against 10mM-Tris/HCl buffer, pH6.0. The dialysis residue was loaded on a Blue Sepharose column (21cm × 1.3cm) and the ketohexokinase activity was eluted with the same buffer. The fractions containing ketohexokinase were pooled and the pH was adjusted to 7.2. They were then concentrated with an Amicon concentrator and stored at −20°C.

Purification of human liver aldolase

Human liver aldolase was prepared by the procedure described by Eagles & Iqbal (1973), with the following modification. After passage through the CM-cellulose 11 column, the protein was precipitated with 60%-satn. $(NH_4)_2SO_4$ and the precipitate chromatographed on a Sephacryl S-200 column (96cm × 3.4cm), which was equilibrated with 10mM-triethanolamine/HCl buffer, pH7.3. The fractions containing aldolase activity were pooled, concentrated with an Amicon concentrator and stored frozen at −20°C.

Results

Purification of ketohexokinase and aldolase

The results for the preparation of ketohexokinase from human liver are shown in Table 1. The final specific activity of the ketohexokinase is similar to that reported for both the ox (Raushel & Cleland, 1977) and rat (Sanchez *et al.*, 1971*a*) liver enzymes. The enzyme appears to be homogeneous when examined by polyacrylamide-gel electrophoresis under both denaturing and non-denaturing conditions (Fig. 1). It is necessary to perform more column-chromatography steps in this preparation than were described for the ox liver enzyme. This is because a greater amount of protein is obtained in the initial step of the human liver preparation than in the ox liver extraction without there being any increase in the total ketohexokinase activity.

The aldolase preparation was examined by polyacrylamide-gel electrophoresis, and is not homogeneous (results not shown), but, using Sephacryl S-200 column chromatography, we have found that the enzyme has a M_r of 150000, which is

Table 1. *Purification of ketohexokinase from human liver*

Purification step	Volume (ml)	Total activity (units)	Total protein (mg)	Specific activity (units/mg)	Yield (%)	Purification (fold)
Crude extract	1450	1571	241692	0.0065	100	
pH step	1358	1272	123688	0.01	81	2
33%-satn.-$(NH_4)_2SO_4$ fraction	1511	954	30774	0.031	61	5
55%-satn.-$(NH_4)_2SO_4$ fraction	43	715	10070	0.071	46	11
Sephacryl S-200 column eluate	92	611	2273	0.27	39	42
DEAE-Sepharose column eluate	108	488	397	1.2	31	185
CM-Sephadex column eluate	88	456	232	1.97	29	302
Chromatofocusing column eluate	44	307	79	3.9	20	600
Blue-Sepharose column eluate	13	141	15	9.4	9	1446

similar to that reported for aldolase from other sources (Peanasky & Lardy, 1958; Gracy *et al.*, 1969). Other physical properties of the enzyme are the same as have been described previously (Eagles & Iqbal, 1973).

These two enzyme preparations were assayed for the following enzymic activities, which could possibly interfere with the system that we are measuring: glycerol-3-phosphate dehydrogenase, polyol dehydrogenase, triosephosphate isomerase, ATPase, glucokinase, phosphofructokinase, alcohol dehydrogenase and lactate dehydrogenase. There was no detectable activity of any of these enzymes in either enzyme preparation.

Physical properties of ketohexokinase

The M_r of the enzyme as determined by column chromatography on Sephadex G-100 and Sephacryl S-200 is 75000. Polyacrylamide-gel electrophoresis in non-denaturing conditions (Davis, 1964) and electrophoresis in the presence of SDS (Weber & Osborn, 1969) both indicate that the enzyme runs as a single protein band (Fig. 1). The protein migrates between malate dehydrogenase (37000) and ovalbumin (43000) on SDS gels and corresponds to a M_r of 39000. Thus the results indicate that the human liver enzyme is a dimer with subunits of M_r 39000. The pI obtained on the Chromatofocusing column for the enzyme is 5.5 (Fig. 2) and is similar to the value of 5.7 obtained for the ox liver enzyme by isoelectric focusing in polyacrylamide gels (Raushel & Cleland, 1977). No metal ions and, in particular, no zinc, magnesium, copper or calcium could be detected in the ketohexokinase preparation by either Energy Dispersion Analysis of X-rays (Woldseth, 1973) or atomic-adsorption spectrophotometry.

The effect of thiol reagents (dithiothreitol, *N*-acetylcysteine and 2-mercaptoethanol) and EDTA on the stability of ketohexokinase was studied by diluting the partially purified enzyme in 20mM-triethanolamine/HCl buffer, pH7.2, containing 10mM effector. We found that the enzyme was stable for at least 48h, after which time the activity decreased in the presence of *N*-acetylcysteine and 2-mercaptoethanol, but to a lesser extent than that which occurred with buffer alone. Ketohexokinase can be inhibited by *p*-chloromercuribenzoate (Adelman *et al.*, 1967; Sanchez *et al.*, 1971*a*), and this indicates that the enzyme has an absolute requirement for a thiol group. However, the results obtained by Adelman *et al.* (1967), using iodoacetate and iodoacetamide, are contradictory and do not support this conclusion. To study whether there is an essential thiol group present in the enzyme, we used not only *p*-chloromercuribenzoate but also *N*-ethylmaleimide, which is considered to be a more specific inactivator of thiol groups

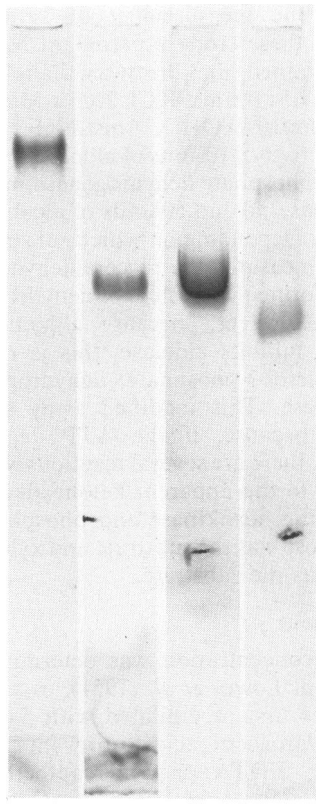

Fig. 1. *Polyacrylamide-gel electrophoresis of ketohexokinase purified from human liver*

The polyacrylamide gels are (1) the pH8.6 system described by Davis (1964) and (2–4) the SDS system described by Weber & Osborn (1969). The samples are (1) and (2) ketohexokinase, (3) ovalbumin marker (M_r 43000) and (4) malate dehydrogenase subunit marker (M_r 37000). For the non-denaturing gel electrophoresis, 60μg of protein was diluted with gel buffer containing 20% sucrose and then loaded on the gel. For the SDS/polyacrylamide-gel electrophoresis, 60μg of protein was incubated overnight with an equal volume of electrophoresis buffer containing 1mM-2-mercaptoethanol. Electrophoresis conditions were as described in the text.

(Morell *et al.*, 1964). We found that both these compounds inhibited ketohexokinase, but that *p*-chloromercuribenzoate was more effective at the same concentration. Partial protection against this inhibition was afforded by the presence of 10mM-fructose, but the other substrate for ketohexokinase, MgATP, and the activator, K^+, did not protect against the inhibition by either *p*-chloromercuribenzoate or *N*-ethylmaleimide.

Kinetic properties of ketohexokinase and aldolase

The K_m and relative $V_{max.}$ values for human liver ketohexokinase and aldolase with various sub-

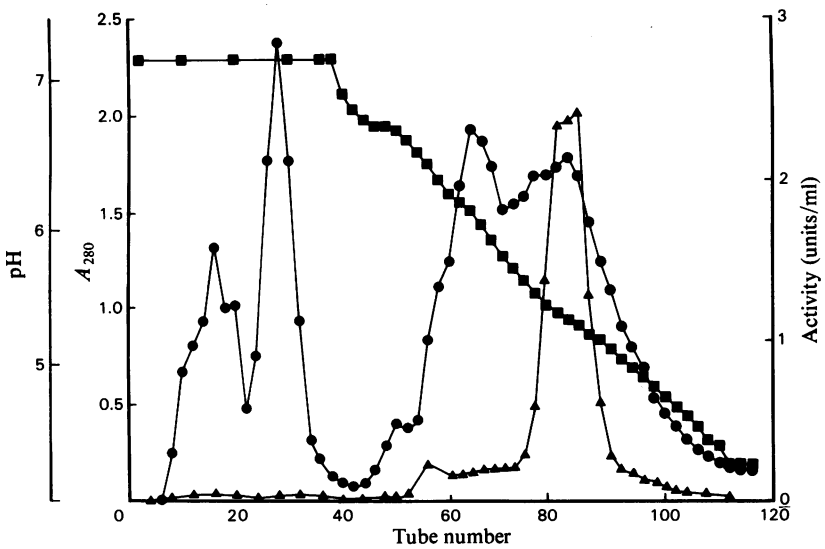

Fig. 2. *Purification of human liver ketohexokinase on a Chromatofocusing column*
The partially purified protein was loaded on the Chromatofocusing column, which had been equilibrated with 25mM-imadazole/HCl buffer, pH7.0. The activity was eluted with Polybuffer, pH4.0. The flow rate was 40ml/h, and 4ml fractions were collected. Protein was monitored by measuring A_{280} (●). Ketohexokinase activity (▲) was measured by the spectrophotometric assay as described in the text. ■, pH.

Table 2. *Apparent kinetic constants for various substrates of ketohexokinase and aldolase from human liver*
Initial rates were calculated from progress curves by using the assay mixtures as described in the text. All assays were performed at 37°C.

Enzyme	Substrate	$K_{m(app.)}$ (mM)	Relative specific activity $[V_{max.(app.)}]$ (%)
Ketohexokinase	D-Fructose	0.86	100
	D-Xylulose	6.0	99
Aldolase	D-Fructose 1,6-bisphosphate	0.0037	100
	D-Fructose 1-phosphate	2.0	99
	D-Xylulose 1-phosphate	0.016	110

strates were determined (Table 2). The K_m value for ketohexokinase with xylulose as the substrate is much greater than that with fructose, but the $V_{max.}$ values are the same. The similar $V_{max.}$ values for the human liver enzyme are in contrast with those found for the enzyme from other species, in which the activity with xylulose was lower (Adelman *et al.*, 1967; Raushel & Cleland, 1977).

For aldolase, the K_m values for fructose 1-phosphate, xylulose 1-phosphate and fructose 1,6-bisphosphate differ considerably, but the $V_{max.}$ values are similar. The K_m values for both fructose 1-phosphate and fructose 1,6-bisphosphate are similar to those reported previously for the human liver enzyme (Gurtler *et al.*, 1971; Eagles & Iqbal, 1973), but the ratio of the $V_{max.}$ values for these two substrates is slightly higher.

Tissue distribution of ketohexokinase and aldolase

The distribution of ketohexokinase and aldolase in various human tissues is shown in Table 3. Ketohexokinase activity is highest in the liver and kidney, with very little activity in the other tissues tested. Aldolase, however, is more widely distributed, with considerable activity in muscle. The activity of ketohexokinase and aldolase in the liver is similar to that previously reported (Heinz *et al.*, 1968).

Coupled reaction sequence of ketohexokinase and aldolase

It has previously been shown that xylitol can be metabolized via a series of reactions in which ketohexokinase and aldolase form glycolaldehyde,

Table 3. *Distribution of ketohexokinase and aldolase in human tissues*
Activities are expressed as means ± S.D. ($n = 5$). The assay mixtures are described in detail in the text.

Enzyme	Substrate	Activity (μmol/min per g of tissue) Liver	Kidney	Brain	Heart	Muscle
Ketohexokinase	D-Fructose	2.8±1.1	2.3±1.2	0.22±0.12	0.14±0.08	0.17±0.08
	D-Xylulose	2.5±1.7	2.1±1.9	0.13±0.18	0.16±0.07	0.12±0.09
Aldolase	D-Fructose 1,6-bisphosphate	11.7±5.4	4.7±1.4	4.6±0.7	4.9±1.8	58±15
	D-Fructose 1-phosphate	4.8±4.4	1.7±0.5	0.22±0.10	0.21±0.03	3.3±2.7
	D-Xylulose 1-phosphate	6.8±6.1	2.2±0.8	0.5±0.17	0.53±0.30	6.1±5.5

which is a direct precursor of oxalate (James *et al.*, 1982; Barngrover *et al.*, 1981). The validity of this pathway has been shown by measuring the formation of the products from xylulose and fructose (James *et al.*, 1982). There are several enzymes with the potential to interfere with this series of reactions (see above), but we could not detect them in our enzyme preparations. The time course for the coupled reaction of ketohexokinase and aldolase with either fructose or xylulose as the initial substrate is shown in Fig. 3. The results are non-linear, which is to be expected for a coupled enzyme system (McClure, 1969; Garcia-Carmona *et al.*, 1981), and the size of the lag can be varied by changing the aldolase concentration.

In addition, we have shown, *in vitro*, the formation of oxalate from xylitol. This sequence of reactions was carried out with enzymes purified from several different sources, namely sheep liver sorbitol dehydrogenase, human liver ketohexokinase and aldolase, yeast aldehyde dehydrogenase, spinach glycollate oxidase and rabbit muscle lactate dehydrogenase. As cofactors, 10mM-ATP, 5mM-NAD^+ and 5mM-FMN were added and, although the incorporation of radioactivity into oxalate from labelled xylitol was low, it was comparable with the amount of oxalate produced from xylitol by isolated hepatocytes (Rofe *et al.*, 1977).

We have measured the effect of a range of metabolites (10mM) on the activity of both ketohexokinase and aldolase. Initial investigations with ketohexokinase were carried out by the spectrophotometric assay, with fructose (25 and 1mM) and xylulose (1mM) as substrates. When there was a change in the activity, the radioactive assay, with fructose as the substrate, was used, as this eliminates any possible effects of the metabolites on the auxiliary enzymes. For aldolase with fructose 1,6-bisphosphate, fructose 1-phosphate and xylulose 1-phosphate (all at $20 \times K_m$) as substrates, the activity can be measured with either alcohol dehydrogenase or glycerol-3-phosphate dehydrogenase as the linking enzyme. If there was any change in the activity of the aldolase assay, the effect was tested by both of these auxiliary enzyme systems.

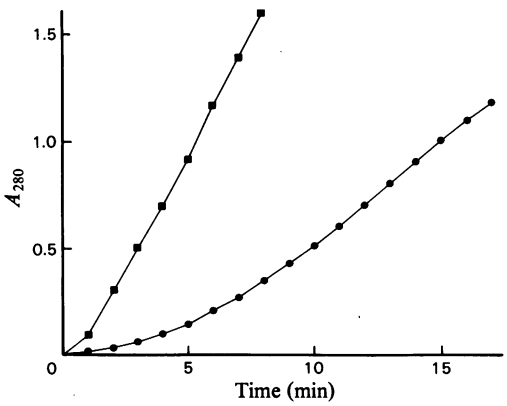

Fig. 3. *Progress curves for reactions of fructose or xylulose with ketohexokinase and aldolase purified from human liver*
The reaction mixtures were essentially as described in the text with the initial substrate concentration being 10mM-fructose (●) or -xylulose (■). Reactions were initiated by the addition of substrate and monitored at 340nm.

These studies showed that, of the intermediates involved in the sequence of reactions which form oxalate from xylulose (James *et al.*, 1982), only glyoxylate had any effect on either ketohexokinase or aldolase. Amino acids and C_5 and C_6 aldoses, ketoses and polyols had no effect on the activity of either enzyme. Of the other compounds tested, Ca^{2+}, ADP and citrate had a significant effect on the ketohexokinase activity. The Ca^{2+} probably competes with Mg^{2+} for the ATP, citrate complexes essential metal ions (e.g. K^+) (Sanchez *et al.*, 1971*b*), and ADP inhibition has been reported previously (Raushel & Cleland, 1977). We also confirmed the last authors' observation of inhibition by fructose 1-phosphate, but the inhibition constant is high and probably has no physiological significance.

Our inhibitor studies with human liver aldolase gave similar results to those described by Woods *et al.* (1970). They used purified rat liver aldolase and

found that several phosphorylated metabolites inhibit the enzymic activity and that, when fructose 1-phosphate (or, as in our studies, xylulose 1-phosphate as well) is a substrate, aldolase is more sensitive to inhibition than when fructose 1,6-bisphosphate is a substrate. The phosphorylated intermediates that caused marked inhibition included glucose 1-phosphate, glucose 6-phosphate, fructose 6-phosphate, ribose 5-phosphate, erythrose 4-phosphate, glycerol 2,3-bisphosphate, ATP, ADP and IMP. Aldolase was also inhibited by ascorbic acid, with a concentration of 1 mM causing 50% inhibition. Consideration of the structure of ascorbic acid and the substrates of aldolase suggest that this effect is due to a steric hindrance by ascorbic acid. It is unlikely, however, that ascorbic acid concentration *in vivo* would be this high, even in association with mega-dose intakes. The other inhibitor of the activity is glyoxylate, but its mechanism of action is unclear.

Discussion

The procedure reported here for the purification of ketohexokinase from human liver results in the isolation of a homogeneous enzyme preparation. Column chromatography and polyacrylamide-gel electrophoresis indicate that the enzyme is a dimer of M_r 75000. Other workers have shown rat liver ketohexokinase to be a monomer of M_r 28000 (Sanchez *et al.*, 1971*a*) and the ox liver enzyme to be a dimer of M_r 56000 (Raushel & Cleland, 1977). However, in their studies with the rat liver enzyme, Sanchez *et al.* (1971*a*) detected at least two protein bands by polyacrylamide-gel electrophoresis which corresponded to the enzymic activity. Thus it may be that the rat liver enzyme can exist in a number of active forms. When we chromatographed rat liver ketohexokinase on Sephacryl S 200 under the same conditions as described for the human liver enzyme, the activity corresponded to an M_r of 75000. One explanation for the discrepancy in the results is that various buffers used in the three studies result in different active forms of the enzyme. We have tried to locate different active forms of the human liver enzyme by using h.p.l.c. with a variety of buffers, but have been unsuccessful. One problem that we found with this work was that, if the preparation was not homogeneous, there were several apparent activity peaks. In our experience, however, ketohexokinase could only be shown to be present in the peaks if the spectrophotometric assay included all the control assays described in the Methods section, or the radiochemical assay was used.

Stability studies on crude extracts of ketohexokinase and the partially purified enzyme give different results and showed that only the purified enzyme could be stabilized by *N*-acetylcysteine or 2-mercaptoethanol. One explanation for these contradictory results is that in the crude extracts, proteinases, which are known to be present in the liver at high concentrations, are released during the extraction procedure and are activated by the thiol reagents or the EDTA. To minimize this problem, phenylmethanesulphonyl fluoride, a proteinase inhibitor, was added to the extraction buffer. With the purified enzyme the presence of thiol groups is required for full catalytic activity, and these groups are most likely to be at the active site of the enzyme (or immediately adjacent to it), because the inactivation could be partially prevented by the substrate, fructose.

Ketohexokinase is most abundant in the liver. The kidney has about half the activity present in the liver, and there are very low activities present in the brain, heart and muscle. These findings agree with the results obtained for the distribution of the enzyme in the rat (Adelman *et al.*, 1967), and our results for the activity in human liver are similar to that found by Heinz *et al.* (1968). In all the human organs tested, the activities with both fructose and xylulose as substrates are very similar, and the liver must be the major organ for xylitol metabolism. It has been claimed by several authors that xylitol can be metabolized by tissues other than liver and kidney, but it is very difficult to ascertain from results of experiments *in vivo* whether one is monitoring the metabolism of xylitol itself or the metabolism of glucose after the liver has converted xylitol into glucose.

Xylulose is the oxidation product of the hepatic NAD-dependent xylitol dehydrogenase, and under normal circumstances this compound can be phosphorylated by xylulokinase to xylulose 5-phosphate (Arsenis & Touster, 1969), which enters the pentose phosphate pathway, and then the glycolytic–gluconeogenic pathway to form glucose, pyruvate and lactate.

The results described above indicate that xylitol can, in addition, be metabolized through xylulose 1-phosphate to glycolaldehyde, a known oxalate precursor. Barngrover & Dills (1983) concluded that the production of xylulose from xylitol is so slow that xylulokinase never becomes saturated and that the xylulose concentration would not reach values sufficient for extensive phosphorylation by ketohexokinase. The relatively high K_m of liver ketohexokinase for xylulose would also support this conclusion. However, xylulose 1-phosphate and glycolaldehyde are produced in isolated hepatocytes treated with xylulose, indicating that this is a minor pathway of xylitol metabolism. The proposed pathway can explain the production of oxalate from xylitol observed in isolated hepatocytes, especially since the amount

of oxalate produced is less than 0.2% of the xylitol utilized (Rofe *et al.*, 1980).

These studies are important in that they provide a biochemical explanation for the clinical observation that a number of patients receiving xylitol, as an intravenous nutrient, developed adverse reactions, including the deposition of calcium oxalate in the kidney, brain, lung and vascular tissues, and this resulted in the death of some of these patients (Thomas *et al.*, 1972; Schröder, 1980). However, the liver is the major site of oxalate production (Liao & Richardson, 1972; Richardson, 1973) and, in xylitol toxicity, it would appear that the oxalate is produced in the liver, and transported to the non-hepatic tissues in which the deposition occurs. It is clear that the production of oxalate from xylitol is a relatively minor pathway, but in situations of excessive xylitol intake, such as parenteral infusion, it does become important.

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References

Adelman, R. C., Ballard, F. J. & Weinhouse, S. (1967) *J. Biol. Chem.* **242**, 3360–3365

Ames, B. N. (1966) *Methods Enzymol.* **7**, 115–118

Arsenis, C. & Touster, O. (1969) *J. Biol. Chem.* **244**, 3895–3899

Barngrover, D. A. & Dills, W. L., Jr. (1983) *J. Nutr.* **113**, 522–530

Barngrover, D. A., Stevens, H. C. & Dills, W. L., Jr. (1981) *Biochem. Biophys. Res. Commun.* **102**, 75–80

Bleakley, P. A., Arora, K. K. & Williams, J. F. (1984) *Biochem. Int.* **8**, 491–500

Byrne, W. L. & Lardy, H. A. (1954) *Biochim. Biophys. Acta* **14**, 495–501

Datta, A. G. & Racker, E. (1961) *J. Biol. Chem.* **236**, 617–623

Davis, B. J. (1964) *Ann. N.Y. Acad. Sci.* **121**, 404–427

Eagles, P. A. M. & Iqbal, M. (1973) *Biochem. J.* **183**, 429–439

Garcia-Carmona, F., Garcia-Canovas, F. & Lozano, J. A. (1981) *Anal. Biochem.* **113**, 286–291

Gracy, R. W., Lacko, A. G. & Horecker, B. L. (1969) *J. Biol. Chem.* **244**, 3913–3919

Gurtler, B., Bally, C. & Leuthardt, F. (1971) *Hoppe-Seyler's Z. Physiol. Chem.* **352**, 1455–1462

Hannett, B. Thomas, D. W., Chalmers, A. H., Rofe, A. M., Edwards, J. B. & Edwards, R. G. (1977) *J. Nutr.* **107**, 458–465

Hauschildt, S. & Brand, K. (1979) *Biochem. Med.* **21**, 55–61

Heinz, F., Lamprecht, W. & Kirsch, J. (1968) *J. Clin. Invest.* **47**, 1826–1832

Hollman, S. & Touster, O. (1957) *J. Biol. Chem.* **225**, 87–102

James, H. M., Bais, R., Edwards, J. B., Rofe, A. M. & Conyers, R. A. J. (1982) *Aust. J. Exp. Biol. Med. Sci.* **60**, 117–122

Landau, B. R. & Wood, H. G. (1983) *Trends Biochem. Sci.* **8**, 292–296

Liao, L. L. & Richardson, K. E. (1972) *Arch. Biochem. Biophys.* **153**, 438–448

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

McClure, W. R. (1969) *Biochemistry* **8**, 2782–2786

Michal, G. & Beutler, H. O. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed), pp. 1314–1319, Academic Press, New York and London

Morell, S. A., Ayers, V. E., Greenwalt, T. J. & Hoffman, P. (1964) *J. Biol. Chem.* **239**, 2696–2705

O'Brien, M. M. & Schofield, P. J. (1980) *Biochem. J.* **187**, 21–30

O'Brien, M. M., Schofield, P. J. & Edwards, M. R. (1983) *Biochem. J.* **211**, 81–90

Peanasky, R. J. & Lardy, H. A. (1958) *J. Biol. Chem.* **233**, 371–373

Pinto, P. V. C., Kaplan, A. & Van Dreal, P. A. (1969) *Clin. Chem.* **15**, 349–360

Raushel, F. M. & Cleland, W. W. (1977) *Biochemistry* **16**, 2169–2175

Richardson, K. E. (1973) *Toxicol. Appl. Pharmacol.* **24**, 530–538

Rofe, A. M., Thomas, D. W., Edwards, R. G. & Edwards, J. B. (1977) *Biochem. Med.* **18**, 440–451

Rofe, A. M., Conyers, R. A. J., Bais, R. & Edwards, J. B. (1979) *Aust. J. Exp. Biol. Med. Sci.* **57**, 171–176

Rofe, A. M., James, H. M., Bais, R., Edwards, J. B. & Conyers, R. A. J. (1980) *Aust. J. Exp. Biol. Med. Sci.* **58**, 103–116

Rognstad, R., Wals, P. & Katz, J. (1982) *Biochem. J.* **208**, 851–855

Sanchez, J. J., Gonzales, N. S. & Pontis, H. G. (1971*a*) *Biochim. Biophys. Acta* **227**, 67–78

Sanchez, J. J., Gonzales, N. S. & Pontis, H. G. (1971*b*) *Biochim. Biophys. Acta* **227**, 79–85

Schröder, R. (1980) *Dtsch. Med. Wochenschr.* **105**, 997–1001

Sluyterman, L. A. Æ. & Elgersma, O. (1978) *J. Chromatogr.* **150**, 17–30

Thomas, D. W., Edwards, J. B., Lawrence, J. R. & Edwards, R. G. (1972) *Med. J. Aust.* **1**, 1238–1246

Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406–4412

Williams, J. F. (1980) *Trends Biochem. Sci.* **5**, 315–318

Williams, J. F., Arora, K. K. & Longenecker, J. P. (1984) *Trends Biochem. Sci.* **9**, 275–277

Woldseth, R. (1973) *X-ray Energy Spectrometry*, Kevex Corp., Burlingame, CA

Woods, H. F., Eggleston, L. V. & Krebs, H. A. (1970) *Biochem. J.* **119**, 501–510