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Purification and Partial Characterization of an Aldo-Keto Reductase from *Saccharomyces cerevisiae*

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A cytosolic aldo-keto reductase was purified from *Saccharomyces cerevisiae* ATCC 26602 to homogeneity by affinity chromatography, chromatofocusing, and hydroxylapatite chromatography. The relative molecular weights of the aldo-keto reductase as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and size exclusion chromatography were 36,800 and 35,000, respectively, indicating that the enzyme is monomeric. Amino acid composition and N-terminal sequence analysis revealed that the enzyme is closely related to the aldose reductases of xylose-fermenting yeasts and mammalian tissues. The enzyme was apparently immunologically unrelated to the aldose reductases of other xylose-fermenting yeasts. The aldo-keto reductase is NADPH specific and catalyzes the reduction of a variety of aldehydes. The best substrate for the enzyme is the aromatic aldehyde *p*-nitrobenzaldehyde (K_m = 46 μM; k_{cat}/K_m = 52,100 s^{-1} M^{-1}), whereas among the aldoses, DL-glyceraldehyde was the preferred substrate (K_m = 1.44 mM; k_{cat}/K_m = 1,790 s^{-1} M^{-1}). The enzyme failed to catalyze the reduction of menadione and *p*-benzoquinone, substrates for carbonyl reductase. The enzyme was inhibited only slightly by 2 mM sodium valproate and was activated by pyridoxal 5′-phosphate. The optimum pH of the enzyme is 5. These data indicate that the *S. cerevisiae* aldo-keto reductase is a monomeric NADPH-specific reductase with strong similarities to the aldose reductases.

The aldo-keto reductases comprise a family of monomeric, NADPH-dependent oxidoreductases with similar physical and chemical properties which catalyze the reduction of aldehydes and ketones to the corresponding alcohol products. The catalyzed reaction favors alcohol formation, whereas the reverse reaction occurs only to a limited extent (56). These reductases have a preferential, but not mutually exclusive, substrate specificity, and this overlapping specificity, together with indeterminate physiological roles, has precluded efforts to derive a more specific nomenclature for these enzymes (20).

Three distinct enzymes which exhibit the typical properties of aldo-keto reductase are known: (i) aldehyde reductase (EC 1.1.1.2), which catalyzes the reduction of a variety of aldehydes, notably uronic acids and some ketones; (ii) aldose reductase (EC 1.1.1.21), which catalyzes the reduction of aldehydes, especially glycolaldehydes and polyolaldehydes, but is less active with uronic acids; and (iii) carbonyl reductase (EC 1.1.1.184), which catalyzes the reduction of quinones, other ketones, and aldehydes to their corresponding alcohols (56). The aldo-keto reductases consist of a single polypeptide chain with a relative molecular mass of between 30,000 and 40,000 for aldehyde and aldose reductases and approximately 30,000 for carbonyl reductases (56). They are found in a wide variety of mammals, birds, reptiles, amphibia, fish, insects, and fungi (18).

Carbonyl as well as aldehyde reductases have been proposed to be involved in the detoxification of reactive compounds in the liver and the brain (20, 56). Aldose reductase catalyzes the first reaction of the polyol pathway in which aldoses are converted by an NADPH-dependent reaction to the corresponding polyalcohols (56). Aldose reductase appears to be strongly involved in the etiology of several human pathologies associated with hyperglycemia (32). Aldose reductase also occurs in xylose-fermenting yeasts such as *Pichia stipitis*, *Candida shehatae*, and *Pachysolen tannophilus* (15, 22). It catalyzes the first step in the xylose metabolic pathway and has been studied because the production of ethanol from xylose is of potential economic value (11). In spite of the fact that the yeast *Saccharomyces cerevisiae* is unable to grow on xylose (3), a number of investigators have found that this yeast can indeed metabolize xylose when another sugar is present (5, 41, 53, 54). However, the rate of xylose utilization is slow, with only 74% of the available xylose being metabolized within 7 days (54). Both glucose- and ethanol-grown cultures were found to possess similar levels of xylose reductase activity (ca. 5 mU/mg of protein), indicating that the enzyme is constitutive and non-glucose repressible (53). Furthermore, many *S. cerevisiae* strains grow on xylulose (21), thereby indicating that the reasons for the failure of *S. cerevisiae* to grow on xylose may be found in the initial steps from xylose to xylulose. The enzymes catalyzing these initial steps in *S. cerevisiae* have not yet been characterized, and an underst anding of the properties of these enzymes could assist in the development of a xylose-fermenting *S. cerevisiae* strain.

The purpose of this investigation was to purify the enzyme catalyzing the conversion of xylose to xylitol in *S. cerevisiae* ATCC 26602 to homogeneity and to characterize its physical, immunological, and kinetic properties. The results indicate that this enzyme is an aldo-keto reductase with properties similar to those of a wide range of aldose reductases found in other microorganisms and animals.

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MATERIALS AND METHODS

Microorganism and growth conditions. Yeast cultures were maintained on yeast peptone dextrose (YPD) agar slants (1% [wt/vol] yeast extract, 2% [wt/vol] peptone, 2% [wt/vol] glucose, 1.5% [wt/vol] agar). *S. cerevisiae* ATCC 26602 was cultured in 9 liters of the above-described medium without agar in a fermentor (VirTis, Gardiner, N.Y.) at 30°C and an aeration rate of 7.5 liters/min. After 24 h of growth, the cells were harvested and washed by centrifugation for 5 min at 5,000 × *g* and 4°C.

P. tannophilus NRRL Y-2460, *C. shehatae* CSIR Y492, *P. stipitis* CSIR Y633, and *Candida utilis* CSIR Y12 were grown in 400 ml of medium containing 1% (wt/vol) yeast extract, 2% (wt/vol) peptone, and 2% (wt/vol) xylose in 3-liter flasks at 30°C. *S. cerevisiae* strains CSIR Y191, 8282 ADE, ATCC 4126, and NRRL Y132 were grown in 400 ml of YPD medium at 30°C. All cells were harvested in the late exponential phase.

Enzyme purification. Lyophilized cells were disrupted either by using a mortar and pestle or by adding glass beads and disrupting the cells in a cell homogenizer (MSK; Braun Melsungen AG, Melsungen, Germany). The extraction buffer consisted of 100 to 150 ml of potassium phosphate (pH 7.4) containing freshly prepared 0.5 mM phenylmethylsulfonyl fluoride (Boehringer Mannheim), 1 μM pepstatin A (Merck Darmstadt), and 50 μM leupeptin (Merck Darmstadt) to inhibit protease activity. The homogenate was centrifuged for 30 min at 48,400 × *g* and 4°C.

The purification was carried out according to a method described by Vander Jagt et al. (52) with the exception that fractions containing aldo-keto reductase activity were pooled and desalted by ultrafiltration with a 50-ml stirred Amicon (Danvers, Mass.) ultrafiltration cell fitted with a YM-10 membrane instead of by gel filtration. All steps were performed at 0 to 4°C.

Protein determination. Protein concentrations were estimated either by measuring the A_{280} or by the bicinchoninic acid method (45) with the Pierce bicinchoninic acid protein assay kit (Rockford, Ill.).

Enzyme assay. Aldo-keto reductase activity was determined routinely at 30°C by monitoring the decrease in A_{340} in an assay mixture containing 50 mM potassium phosphate buffer (pH 7.4), 115 mM xylose, 0.12 mM NADPH, and 10 mM 2-mercaptoethanol (44). The determination of the effects of pH and sodium valproate (Merck Darmstadt) on enzyme activity was carried out in a similar manner except that 10 mM DL-glyceraldehyde was used as a substrate. For assays at different pH values, 50 mM sodium citrate (pH 3 to 5.4) and 50 mM potassium phosphate (pH 6 to 8) buffers were used. The effect of sodium valproate on enzyme activity was determined in 50 mM sodium citrate buffer (pH 5), whereas the effect of pyridoxal 5′-phosphate (Merck Darmstadt) (0.25 or 0.5 mM) on aldo-keto reductase activity was determined for 60 min at room temperature in the dark as described by Morjana et al. (39). The appropriate blanks to correct for nonspecific oxidation of NADPH were prepared for each assay. The Michaelis kinetic parameters, K_m and k_{cat}, were determined by fitting data directly to the rate equation with SAS software (SAS Institute Inc.). One unit of enzyme activity was defined as the amount of enzyme catalyzing the oxidation of 1 μmol of NADPH per min under the above-described assay conditions.

PAGE. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on a 10 to 20% gradient gel by the method of Laemmli (35). Protein bands were visualized with Coomassie brilliant blue G (Sigma Chemical Co., St. Louis, Mo.) or by silver staining (46). Standard proteins (Boehringer Mannheim) used for estimation of molecular mass were α_2-macroglobulin (M_r = 170,000), β-galactosidase (M_r = 116,400), fructose-6-phosphate kinase (M_r = 85,200), glutamate dehydrogenase (M_r = 55,600), aldolase (M_r = 39,200), triose phosphate isomerase (M_r = 26,600), trypsin inhibitor (M_r = 20,100), and lysozyme (M_r = 14,300).

Molecular mass determination by size exclusion chromatography. The molecular mass of the aldo-keto reductase was also determined under nonreducing conditions by size exclusion chromatography (1) using a Toyopearl HW-55F (Tosohaas, Stuttgart, Germany) column (645 by 8 mm) attached to a fast protein liquid chromatography system. A 50 mM phosphate buffer (pH 7.5) containing 0.3 M NaCl and 5 mM 2-mercaptoethanol was used as the running buffer at a flow rate of 0.25 ml/min. The column was calibrated with the following standard proteins (Boehringer Mannheim): ferritin (M_r = 450,000), aldolase (M_r = 240,000), catalase (M_r = 158,000), hen egg albumin (M_r = 45,000), chymotrypsinogen A (M_r = 25,000), and cytochrome *c* (M_r = 12,500).

Amino acid analysis. Approximately 500 μg of enzyme was precipitated with 30% (wt/vol) trichloroacetic acid, and the precipitate was washed with ice-cold acid acetone (0.1% [vol/vol] concentrated HCl in acetone). The amino acid composition of the dried acetone powder was analyzed by ion-exchange chromatography (7) on an amino acid high-performance liquid chromatography system (Waters, Milford, Mass.). The compositional relatedness between aldo-keto reductase from *S. cerevisiae* and aldo-keto reductases from various other sources was assessed by the method of Metzger et al. (37).

N-terminal sequence analysis. The N-terminal sequence of the purified protein was determined by analyzing the dried acetone powder by a procedure described by Hewick et al. (25) and modified by Brandt et al. (13).

Immunochemical methods. Antiserum to purified aldo-keto reductase was raised in Californian rabbits (29), and immunoblotting was performed as described by Towbin et al. (50). Briefly, proteins were resolved by SDS-PAGE and were then electrophoretically transferred onto a Hybond C nitrocellulose filter. The blotted proteins were identified immunochemically by the sequential addition of anti-aldo-keto reductase serum followed by goat anti-rabbit immunoglobulin G antibodies conjugated to alkaline phosphatase, nitroblue tetrazolium salt, and 5-bromo-4-chloro-3-indolyl-phosphate (Merck Darmstadt). Antiserum raised against purified aldose reductase from *P. tannophilus* (10) was kindly provided by P. L. Bolen.

TABLE 1. Purification of aldo-keto reductase from *S. cerevisiae* ATCC 26602

Step	Amt of total protein (mg)	Total activity (U)	Specific activity (U/mg)	Yield (%)	Purification (fold)
Supernatant	1,274	4.15	0.0032	100	1
Red Sepharose	12.6	5.39	0.428	130	134
Chromatofocusing		3.05		74	
Hydroxylapatite	0.8	3.11	3.90	75	1,218

RESULTS AND DISCUSSION

The protocol for purification of aldo-keto reductase from *S. cerevisiae*, summarized in Table 1, provided a 1,218-fold purification of enzyme with a 75% yield to a final specific activity of 3.90 U/mg of protein with D-xylose as the substrate. This compared favorably with the purification of aldose reductase from human tissues by a similar procedure (52). The purified enzyme was found to be homogeneous by SDS-PAGE (Fig. 1).

Analysis of the enzyme by gel electrophoresis in the presence of SDS (Fig. 1) revealed one band with an M_r of 36,800 ± 1,000 (n = 6). Size exclusion chromatography on Toyopearl HW-55F resulted in the elution of the enzyme activity as a symmetrical peak corresponding to an M_r of approximately 35,000. This result, taken together with that of SDS-PAGE, indicates that the *S. cerevisiae* aldo-keto reductase is a monomeric enzyme. Xylose reductases have been reported to be either monomeric (24) or dimeric (10, 42, 55) in a number of yeasts. The evaluated M_r for this enzyme is, however, in the same range (30,000 to 40,000) as those reported for several other monomeric aldo-keto reductases (56).

The amino acid composition of aldo-keto reductase from *S. cerevisiae* (data not shown) was found to contain a high proline content (6%), typical for aldo-keto reductases (14, 38). The results of compositional relatedness analysis (to determine the difference index [DI], where 0 represents two identical proteins and 100 represents two proteins with no identical amino acids [37]) of aldo-keto reductase from *S. cerevisiae* and aldo-keto reductases from various other sources indicated that the aldo-keto reductase from *S. cerevisiae* is most closely related to the aldose reductase from human kidney (DI = 7.38 [2]). It is also closely related to the xylose reductase from *P. stipitis* (DI = 8.00 [24]) and the aldose reductases in rabbit muscle (DI = 8.16 [17]), *C. shehatae* (DI = 8.33 [26]), *P. tannophilus* (DI = 8.42 [10]) and human muscle (DI = 9.55 [38]). DI analysis indicated that the aldo-keto reductase of *S. cerevisiae* was less closely related to aldehyde (DIs ranging from 10.33 to 21.11 [2, 30, 49]) and carbonyl (DI > 16 [27]) reductases.

Homology of the first 13 residues of the N-terminal sequence apparently exists among aldose reductases from various sources (Fig. 2). Each N-terminal sequence of aldose reductase from various sources contains a highly conserved LNNG region (positions 6 to 9, according to *S. cerevisiae* aldo-keto reductase numbering). The asparagine residue at position 8 (*S. cerevisiae* aldo-keto reductase numbering) in this motif is

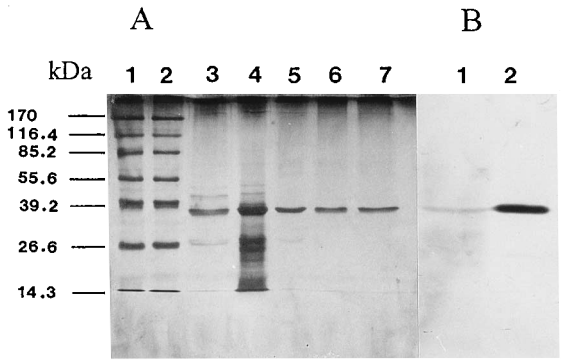

FIG. 1. (A) SDS-PAGE (10% acrylamide) shows a homogeneous enzyme preparation after the final purification step, namely hydroxylapatite (lanes 6 and 7). Lanes 1 and 2, calibration proteins; lane 3, proteins which were present after the red Sepharose purification step; lane 4, proteins present after the desalting step (more protein was loaded in lane 4 than in lane 3, resulting in more and thicker bands); lane 5, proteins present after the chromatofocusing step. The protein bands were visualized by silver staining (46). (B) Western blotting (immunoblotting) of aldo-keto reductase illustrates the specificity of the antiserum which was raised against the purified aldo-keto reductase. Lane 1, crude extract of *S. cerevisiae*; lane 2, purified aldo-keto reductase.

	1	2	3	4	5	6	7	8	9	10	11	12	13
(a)	S	**S**	L	**V**	**T**	**L**	**N**	**N**	**G**	**L**	**K**	**M**	**P**
(b)	M	A	**S**	R	L	L	**L**	**N**	**N**	**G**	A	**K**	**M**	**P**
(c)	S	P	S	P	I	P	A	F	K	**L**	**N**	**N**	**G**	**L**	E	**M**	**P**
(d)				**T**	L	N	Y	Y	**T**	**L**	**N**	**N**	**G**	R	**K**	I	**P**
(e)				M	A	**S**	**H**	L	E	**L**	**N**	**N**	**G**	T	**K**	**M**	**P**
(f)			M	A	A	**S**	C	V	L	**L**	H	T	**G**	Q	**K**	**M**	**P**
(g)					V	G	T	**T**	**T**	**L**	N	T	**G**	A	S	L	E
(h)						P	S	I	K	L	**N**	S	**G**	Y	D	**M**	**P**
(i)	M	P	A	T	L	H	D	S	T	K	I	L	S	L	**N**	T	**G**	A	Q	I	**P**
(j)		M	S	S	G	I	H	V	A	L	V	T	G	G	**N**	K	**G**	I	G	L	A

FIG. 2. Alignment of N-terminal amino acid sequences of aldo-keto reductases. a, *S. cerevisiae* aldo-keto reductase (this paper); b, human placenta aldose reductase (9); c, *C. shehatae* aldose reductase (26); d, *P. tannophilus* aldose reductase (10); e, rat lens aldose reductase (16); f, human liver aldehyde reductase (8); g, *S. salmonicolor* aldehyde reductase (30); h, *P. stipitis* xylose reductase (24, 47); (i) GCY (yeast gene product) (40); (j) human placenta carbonyl reductase (57). Boldface type is used to indicate identical residues. The numbering used is that of *S. cerevisiae* aldo-keto reductase.

replaced by a threonine in the aldehyde reductases from human liver and *Sporobolomyces salmonicolor*. The proline residue in the 13th position is also well conserved, and lysine and methionine residues in the 11th and 12th positions, respectively, are well conserved in some reductases. The conserved glycine in position 9 and the proline in position 13 are thought to compose part of the consensus sequence for the "Rossmann fold" involved in nucleotide binding (16).

A gene encoding a protein (GCY) homologous to mammalian aldo-keto reductases has been isolated from *S. cerevisiae* (36, 40). Its N-terminal sequence and its kinetic properties, however, indicate that this enzyme is probably a carbonyl reductase not related to the aldo-keto reductase described here.

Analysis of purified *S. cerevisiae* aldo-keto reductase on immunoblots developed with anti-*S. cerevisiae* aldo-keto reductase revealed a single protein (Fig. 1B). No bands were detected when preimmune serum was used (data not shown). The cross-reactivity of the antiserum with crude extracts of *S. cerevisiae* CSIR Y 191, *S. cerevisiae* 8282 ADE, *S. cerevisiae* ATCC 4126, and *S. cerevisiae* NRRL Y 132 grown in YPD broth was examined (data not shown). Aldo-keto reductase activity in these strains was found to be insignificant ($\leq$0.9 mU/mg). Weak bands indicated the presence of an aldo-keto reductase in these *S. cerevisiae* strains. However, a number of other bands were also observed, indicating nonspecific cross-reactivity possibly as a result of the use of polyclonal antiserum.

When *S. cerevisiae* ATCC 26602 was grown in YPD broth containing 3% NaCl to test whether aldo-keto reductase activity could be induced by NaCl as was found for renal medullary cells (6), no increase in the specific activity of the aldo-keto reductase relative to the activity found in *S. cerevisiae* which was grown in YPD broth without NaCl (3 to 8 mU/mg) was found. This finding was confirmed by immunoblotting, which showed no marked increase in the intensity of the aldo-keto reductase band (data not shown) and which indicates that aldo-keto reductase activity is not osmotically regulated in *S. cerevisiae*.

No cross-reactivity between antiserum raised against *S. cerevisiae* aldo-keto reductase and crude extracts of xylose-grown cultures was observed (data not shown), in spite of the fact that aldo-keto reductase specific activity was found in *P. stipitis*, *C. shehatae*, and *P. tannophilus* (between 20 and 165 mU/mg) and *C. utilis* (2.1 mU/mg). Similarly, antiserum raised against *P. tannophilus* NRRL Y-2460 failed to react with a crude extract of *S. cerevisiae* ATCC 26602 on immunoblots (data not shown), thereby indicating that the aldo-keto reductase of *S. cerevisiae* is immunologically unrelated to that found in xylose-fermenting yeasts. Poor interspecies cross-reactivity of anti-aldehyde reductase antibody has, however, been reported (56). This feature might also be characteristic of anti-aldose reductase antibodies.

Optimal reduction of DL-glyceraldehyde by purified *S. cerevisiae* aldo-keto reductase was observed at pH 5 (Fig. 3). At pH 3, 87% of the activity remained, whereas at pH 8 the enzyme activity was only 10% of the value at pH 5. The preference for a slightly acidic pH optimum is typical of aldose reductases (16), although the optimum pH of this aldo-keto reductase is apparently 1 to 2 pH units lower than has previously been observed for other yeast (10, 12, 33, 55) and some mammalian (38, 49) aldose reductases. The optimum pH reported here for the *S. cerevisiae* enzyme is, however, similar to the values of 4.8 and 5.6 reported for the pig and rabbit lens aldose reductases, respectively (14, 48).

The broad substrate specificity of purified *S. cerevisiae* aldo-keto reductase is apparent from Table 2. The aldo-keto reductase discriminates among substrates almost entirely in the binding step, since k_{cat} values are essentially independent of the substrate. The high affinity of the enzyme for *p*-nitrobenzaldehyde is typical for all aldo-keto reductases (51). In general, the enzyme preferred aromatic or aliphatic aldehydes to aldose sugars. Its substrate specificity, as shown by increasing K_m values, was as follows: *p*-nitrobenzaldehyde, DL-glyceraldehyde, D-glyceraldehyde, L-glyceraldehyde, D-glucose, D-xylose, and L-arabinose. This order of substrate affinity is in agreement with observations reported for aldose reductases from various mammalian sources (2, 6, 14) and the yeasts *P. stipitis* (55) and *P. tannophilus* (19). On the one hand, the *S. cerevisiae* aldo-keto reductase had a lower affinity for the aromatic and aliphatic aldehydes compared with the values observed for mammalian aldose reductases, whereas on the other hand, the yeast aldo-keto reductase had a greater affinity for glucose. Arguments that the open-chain aldehyde form of glucose is the true physiological substrate have been made, and a K_m of 0.66 μM has been calculated for aldose reductase when reacted with this form of glucose (28). Accounting for the aldehyde form of glucose and galactose leads to very low corrected K_m values

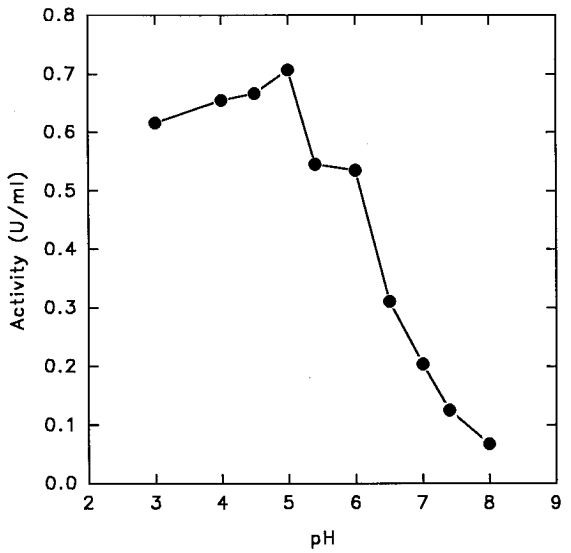

FIG. 3. The effect of pH on the activity of purified aldo-keto reductase of *S. cerevisiae* ATCC 26602. The activity of the purified enzyme was measured as described in Materials and Methods.

and high k_{cat}/K_m values in the case of the rat testis aldose reductase (31). Indeed, Grimshaw (23) has used aldose reductase to measure directly the rate of ring opening of D-glucose, and his results further show that aldose reductase does not itself catalyze the ring-opening reaction. A correlation between the local concentration of glucose in a tissue, the rate of ring opening, and the accumulation of sorbitol has not been made, and there is no direct evidence to date that aldose reductase in vivo uses the acyclic form of D-glucose as a substrate.

The catalytic efficiency (k_{cat}/K_m) of the *S. cerevisiae* aldo-keto reductase was lower than those of the mammalian aldose reductases on aromatic and aliphatic aldehydes, whereas this rate was higher on the aldoses (17, 22, 52). The *S. cerevisiae* aldo-keto reductase failed to exhibit activity on D-glucuronate, an anionic substrate used to characterize aldehyde reductase (22). Aldose reductases are generally less active with uronic acids than are aldehyde reductases (56). In addition, sodium valproate, which has been shown to inhibit aldehyde reductase (17, 38, 51), is ineffective against the purified *S. cerevisiae* enzyme at a concentration of 1 mM. Slight inhibition (16%) was, however, observed with 2 mM sodium valproate. No activity was observed with the quinones menadione and *p*-benzoquinone, which are good substrates for carbonyl reductase (51).

TABLE 2. Michaelis constants and maximal reaction velocities for substrates of *S. cerevisiae* aldo-keto reductase[a]

Substrate	k_{cat} (SEM)[b] (s^{-1})	K_m (SEM) (mM)	k_{cat}/K_m ($mM^{-1} \cdot s^{-1}$)
p-Nitrobenzaldehyde	2.37 (0.05)	0.046 (0.004)	52.1
DL-Glyceraldehyde	2.58 (0.13)	1.44 (0.26)	1.79
D-Glyceraldehyde	3.32 (0.18)	1.57 (0.29)	2.12
L-Glyceraldehyde	2.50 (0.14)	6.38 (1.05)	0.39
D-Xylose	3.37 (0.18)	27.90 (4.51)	0.12
L-Arabinose	1.61 (0.05)	32.63 (2.81)	0.049
D-Glucose	0.71 (0.02)	9.34 (0.79)	0.076
NADPH		0.013 (0.002)	

[a] Enzyme activities were assayed in triplicate with 50 mm potassium phosphate buffer (pH 5.0) containing 0.12 mM NADPH and 10 mM 2-mercaptoethanol, and K_m and k_{cat} values were determined by using a direct fit of the Michaelis-Menten rate equation.

[b] SEM, standard error of the mean.

The K_m value of *S. cerevisiae* aldo-keto reductase with NADPH as a cofactor was similar to values reported for mammalian aldose reductases (2, 22) and aldose reductases from the yeasts *P. tannophilus* (19) and *P. stipitis* (55). No activity, however, was detected with NADH as a cofactor, which is somewhat surprising, since aldose reductases often are able to utilize both cofactors (51), although the aldose reductases generally have a greater affinity for NADPH than NADH in mammalian tissues (58) and in yeasts (42, 55). This finding is not that unusual, however, in the case of yeasts. Bruinenberg et al. (15) have reported the presence of NADPH-dependent xylose reductases exhibiting no activity with NADH in *C. utilis* and certain strains of *Candida tenuis*. Insignificant reverse reaction rates with 200 mM xylitol (<5% of the forward reaction rates with 50 mM xylose), typical of an aldo-keto reductase, were found.

The aldo-keto reductase was activated 40% by 250 μM pyridoxal 5′-phosphate after 30 min and 35% by 125 μM pyridoxal 5′-phosphate after the same amount of time. During this period, the control activity decreased by 10%. This activation by pyridoxal 5′-phosphate, possibly as a result of modification of an essential lysine in the coenzyme binding site, is in agreement with previous findings for aldose reductase from human muscle (39).

The physiological function of this aldo-keto reductase is uncertain. The diversity of aldehyde structures reduced by the aldose reductases has indicated that they are involved in the detoxification of both endogenous and exogenous aldehydes (22). In pentose-fermenting yeasts, aldose reductase catalyzes the first step in the metabolism of D-xylose (43). D-Xylose is converted to xylitol by aldose reductase, and the xylitol is oxidized further via D-xylulose through the pentose phosphate and Embden-Meyerhof pathways. The inability of *S. cerevisiae* to grow on xylose (54) indicates that the aldo-keto reductase activity is unlikely to function in the catabolism of xylose (53). Furthermore, a role in osmoregulation, as found for aldose reductases in PAP-HT25 cells (rabbit renal medullary cells in tissue culture) (6) and in the barley embryo (4), is unlikely, as the aldo-keto reductase levels in *S. cerevisiae* were not changed by osmotic stress.

The construction of a recombinant *S. cerevisiae* strain able to grow and metabolize xylose to xylitol (24, 34, 47) has been attempted by cloning the aldose reductase of *P. stipitis*. The presence of an aldo-keto reductase in *S. cerevisiae* could have potential application in the conversion of xylose to xylitol (a potentially important nonnutritive sweetener [24]). However the NADPH specificity of the enzyme would make it unsuitable for the development of a recombinant *S. cerevisiae* strain able to produce ethanol, as only yeasts with NADH-specific xylose reductase activity are able to ferment xylose (15).

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