Purification and Properties of an NADPH-Aldose Reductase (Aldehyde Reductase) from *Euonymus japonica* Leaves

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ABSTRACT

The enzyme aldose (aldehyde) reductase was partially purified (142-fold) and characterized from *Euonymus japonica* leaves. The reductase, a dimer, had an average molecular weight of 67,000 as determined by gel filtration on Sephadex G-100. The enzyme was NADPH specific and reduced a broad range of substrates including aldoses, aliphatic aldehydes, and aromatic aldehydes. Maximum activity was observed at pH 8 in phosphate and Tris-HCl buffers and at pH 8.6 to 9.0 in glycine-NaOH buffer using DL-glyceraldehyde or 3-pyridinecarboxaldehyde as substrate. NADP was a competitive inhibitor with respect to NADPH with a K_i of 60 micromolar. Glycerol was an uncompetitive inhibitor to DL-glyceraldehyde ($K'_i = 460$ millimolar). The *Euonymus* enzyme was inhibited by sulfhydryl inhibitor, phenobarbital, and high concentrations of Li_2SO_4. Pyrazol and metal chelating agents inhibited the enzyme slightly. Enzyme activity was detected in the leaves and berries of *Celastrus orbiculatus* and several species of *Euonymus*. Probable function of this enzyme is to reduce D-galactose to galactitol, a characteristic metabolite in phloem sap of members of the Celastraceae family.

The biosynthesis of polyols in higher plants appears to be through the reduction of aldose phosphates by NADPH to the corresponding polyol phosphates. Three enzymes have been characterized: aldose-6-P reductase (EC 1.1.1.200) reduces D-glucose-6-P to sorbitol-6-P (16, 20), D-mannose-6-P reductase (24) converts D-mannose-6-P to mannitol-1-P, and D-ribose-5-P reductase (21) which reduces D-ribose-5-P to D-ribitol-5-P.

In other organisms, there are several possible reactions that have been reported for polyol synthesis, *e.g.* the reduction of aldoses or ketoses by either NADH or NADPH, and the reduction of aldose phosphates or ketose phosphates by either NADH or NADPH (21).

Several NADPH-linked aldehyde reductases (alcohol: NADP oxidoreductase, EC 1.1.1.2) have been isolated from mammalian tissues. The broad and overlapping substrate specificities of these enzymes have resulted in confusing nomenclature (13). These enzymes reduce aliphatic aldehydes, aromatic aldehydes, and aldo sugars to their alcohol forms. The enzyme aldose reductase (EC 1.1.1.21), which is responsible for sorbitol synthesis, falls into the general category of aldehyde reductases, but it has a higher affinity for aldoses than other aldehyde reductases (8).

Galactitol (dulcitol) is a characteristic compound of the Celastraceae and a major carbohydrate in the phloem sap of this family (3). Despite the fact that several horticultural crops contain large amounts of galactitol, its metabolism in plants is not known. The objective of this research is to purify and characterize the enzyme responsible for galactitol biosynthesis in *Euonymus*

sp. as a first step towards understanding the role of these compounds in plants.

MATERIALS AND METHODS

Chemicals. Most chemicals were purchased from Sigma Chemical Co. Glyoxal, erythrose, 3-pyridylcarbinol, and butyraldehyde were obtained from Aldrich Chemical Co. Protein standards for gel filtration (low mol wt) were from Pharmacia, Inc. Bovine γ-globulin and all electrophoresis reagents were obtained from Bio-Rad laboratories. All buffers were adjusted to the desired pH at room temperature. NADPH and NADH were dissolved in 1% $NaHCO_3$ and made immediately prior to use. NADP was dissolved in glass distilled H_2O. NADPH and NADP concentrations were determined from the molar extinction coefficients of 6.22 $\times 10^3$ at 340 nm and $18.0 \times 10^3 cm^{-1} M^{-1}$ at 260 nm, respectively. Water insoluble chemicals were dissolved in absolute methanol and dilutions were made in H_2O thereafter. The presence of 2.5% methanol in the reaction mixture showed no effect on enzyme activity.

Plant Material. *Euonymus japonica* leaves used for enzyme purification were obtained from plants grown in a greenhouse under long day conditions. Other *Euonymus* sp. plants were grown outdoors and leaves were collected during the month of August and were kept at −65°C until they were used.

Enzyme Purification. All purification steps were conducted in a cold room at 2 to 4°C. *E. japonica* fully expanded mature leaves (150 g) were homogenized in 500 ml 0.2 M Tris-HCl buffer (pH 8.0), containing 1 mM DTT, 10 mM isoascorbate, and 25 g insoluble PVP. PVP was prewetted with buffer for 1 h prior to homogenization. Leaves were homogenized first in a Sorval Omni-mixer for four 20-s intervals at full speed, followed by additional four 20-s intervals at full speed in a Polytron (Brinkman) homogenizer. The homogenate was squeezed through six layers of cheesecloth and centrifuged at 4,068g for 20 min. The supernatant was centrifuged for 30 min at 23,430g and the pellet was discarded. To the supernatant, solid $(NH_4)_2SO_4$ was added slowly with continuous stirring to give 40% saturation (24.3 g/100 ml). Stirring was continued for additional 20 min and the precipitate was removed by centrifugation at 23,430g for 30 min. The supernatant was made 75% saturation with solid $(NH_4)_2SO_4$ (24.5 g/100 ml) as described above and the precipitate (40–75% fraction) was collected by centrifugation at 23,430g for 30 min. The pellet was dissolved in a minimum volume of 10 mM Tris-HCl buffer (pH 7.2) containing 5 mM 2-mercaptoethanol (buffer A) and dialyzed overnight against three changes (1 L each) of buffer A.

The dialyzed 40 to 75% fraction was centrifuged at 26,890g for 30 min and the pellet was discarded. This fraction was applied to a Sephacryl S-200 column (2.5 × 90 cm) and eluted with 50 mM Tris-HCl buffer (pH 7.2) containing 5 mM 2-mercaptoethanol and 0.02% NaN_3 (buffer B) at a flow rate of 40 ml/h. Active

fractions were pooled and concentrated using an Amicon PM-10 ultrafiltration membrane (Amicon Corporation).

The Sephacryl concentrate was applied to a DEAE-Sephacel column (2.6 × 50 cm) previously equilibrated with buffer B. The column was washed with buffer B until no protein was detected in the eluate (by monitoring UV A at 280 nm) and the enzyme was eluted with a linear gradient of 0 to 1 M NaCl in buffer B (1 L each) at a flow rate of 40 ml/h. Active fractions were pooled and concentrated as described above.

The DEAE Sephacel concentrate was applied to a Sephadex G-100 column (2.5 × 90 cm) previously equilibrated with 20 mM Tris-HCl buffer (pH 7.2) containing 5 mM 2-mercaptoethanol and 0.02% NaN3 (buffer C). The enzyme was eluted with buffer C at a flow rate of 17 ml/h and active fractions were pooled and concentrated as described above.

The Sephadex concentrate was applied to a Sigma Reactive Red 120-Agarose column (1 × 5 cm) which had been equilibrated with buffer C. After 1 h at 0 flow, the column was washed with buffer C until no protein was detected in the eluate. The enzyme was then eluted with 0.35 mM NADPH in buffer C. Active fractions were pooled and dialyzed overnight against 1 L of buffer A and concentrated as described above. The enzyme was stored in small aliquots at −65°C until used for assays. Unless otherwise indicated, this fraction was used for all studies reported in this paper.

Protein Determination. Protein concentrations were determined according to the method of Bradford (4), using bovine γ-globulin as a standard.

Electrophoresis. PAGE was carried out in 7% w/v polyacrylamide gel at pH 8.9 according to Davis (10). Gels were stained for protein according to Reisner (23). Gels were stained for the reductase activity by incubating the gel first in 0.1 M glycine-NaOH buffer (pH 9.5) for 1 h followed by a mixture consisting of 0.4 mg/ml NADP, 0.39 mg/ml 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), 0.04 mg/ml phenazine methosulfate, and either glycerol, propylene glycol (1,2-propanediol), galactitol, or 3-pyridylcarbinol (200 mM final concentration). Gels were kept in the dark at 37°C for 20 to 30 min then washed in dH$_2$O and stored in 7% acetic acid.

Molecular Weight Determination. The reductase mol wt was estimated by gel filtration on a Sephadex G-100 column (2.6 × 45 cm), calibrated with a mixture of known mol wt protein standards (Pharmacia Instruction Manual). The column was equilibrated and eluted with 50 mM Tris-HCl buffer (pH 7.2) containing 0.1 M NaCl at a flow rate of 12 ml/h. The standard enzyme assay was used to detect the reductase activity in the eluate.

SDS-PAGE (11% gel) was performed as described per Sigma Technical Bulletin No. MWS-877L.

Enzyme Assay. Aldose reductase activity was assayed by recording the decrease of NADPH absorption at 340 nm in a Perkin-Elmer model 552 spectrophotometer at 25°C. The standard assay contained in a total volume of 1 ml: 90 mM sodium phosphate buffer (pH 8.0), 110 μM NADPH, enzyme (affinity fraction), and 10 mM DL-glyceraldehyde. Unless otherwise specified, enzyme was incubated with NADPH for 2.5 min prior to addition of substrate to start the reaction. The reference cuvette contained all reagents except the substrate.

One unit of enzyme activity was defined as the amount of enzyme catalyzing the oxidation of 1 μmol NADPH/min at 25°C. Specific activity was expressed as units/mg protein.

RESULTS AND DISCUSSION

Enzyme Purification. The results of a typical purification procedure are shown in Table I. The reductase was purified 142-fold with a 13% yield using DL-glyceraldehyde as the substrate. The partially purified enzyme wa stable at −65°C for more than

1 year. At 1°C, the enzyme lost approximately 15% of its activity after 2 weeks. The enzyme was retained by affinity material and eluted with NADPH suggesting that the protein possesses the dinucleotide fold (27).

When 31 μg of the final preparation was subjected to polyacrylamide disc electrophoresis, a single band of protein was visible. This band corresponded to enzyme activity using either glycerol, 3-pyridylcarbinol, propylene glycol, or galactitol (Fig. 1), suggesting that a single enzyme was responsible for the reduction of these substrates. To further test the presence of a single reductase, DL-glyceraldehyde, D-galactose, and 3-pyridinecarboxaldehyde were used as substrates at each step of the purification procedure, and the ratio of these substrate activities remained constant (data not shown). In addition, the pH profiles for two substrates were identical (Fig. 2). These results indicate the presence of a single enzyme with broad substrate specificity and is similar to aldose and aldehyde reductases isolated from mammalian sources (15, 31). The product of the reaction mixture using D-galactose as a substrate was identified as galactitol by GC and HPLC.

Effect of pH. The effect of the assay mixture pH on the reduction of DL-glyceraldehyde and 3-pyridinecarboxaldehyde using three different buffer systems is shown in Figure 2. The optimum pH range for both substrates was similar and depended on the buffer used in the assay. Maximum activity was observed at pH 8 in Na-phosphate and Tris-HCl buffers and at pH 8.6 to 9.0 in glycine-NaOH buffer. In addition, maximum velocity in Tris-HCl buffer was less than that in phosphate or glycine-NaOH buffer. Similar responses to the pH of the assay mixture have been reported for aldehyde reductase from yeast (*Rhodotorula* sp.) (30) and pig brain (29). The pH optimum for the *Euonymus* enzyme was generally higher than other reductases (5.2 for indole-3-acetaldehyde reductase from cucumber (6), 5.6 for fatty aldehyde reductase from *Brassica oleraceae* (18), 7.0 for aldose reductase from *Vicia faba* (19), and 6.5 for several mammalian aldose reductases (22)).

Molecular Weight. The elution volume for the enzyme was the same as BSA in Sephadex G-100, mol wt 67,000 (Fig. 3A). PAGE (31 μg enzyme protein/gel) in the presence of SDS gave one dark band corresponding to a mol wt of 36,000 (Fig. 3B) and three lightly stained bands below it (Figure not shown). This finding indicates that the aldose (aldehyde) reductase from *E. japonica* is a dimer. Most of the reported mol wt for aldose or aldehyde reductases, a monomer, are in the range of 30,000 to 40,000 (8, 14, 31). However, aldose reductase isolated from *Rhodotorula* (26) and calf liver (1) had a mol wt of 61,000.

Substrate Specificity. The K_m values for several substrates using NADPH as a cofactor are shown in Table II. The enzyme was specific for NADPH and no activity was detected with 110 μM NADH and 10 mM DL-glyceraldehyde. The enzyme reduced a broad range of compounds ranging from aldoses to aliphatic and aromatic aldehydes. The K_m values for aldoses increased with increasing number of carbon atoms. Activities with five-carbon aldoses varied, depending on the sugar; D-ribose was inactive, whereas L-arabinose had a lower K_m value than L-xylose. D-Lyxose was slightly active (Table III). D-Glucose was completely inactive as a substrate, but D-galactose had a K_m value of 227 mM. Most of the aldose and aldehyde reductases isolated from mammalian sources have reacted with D-glucose and D-galactose (12, 15, 28). The high K_m values for hexoses may be caused by the requirement for a free aldehyde group in the substrate (15) which would explain the low K_m values for DL-glyceraldehyde and D-erythrose (Table II).

The inability of the *E. japonica* enzyme to reduce D-glucose and D-ribose could be explained by the requirement for a free aldehyde group in the substrate. Hayman and Kinoshita (15) reported that the K_m for D-glucose was three times higher than

Table I. *Purification of Aldose (Aldehyde) Reductase from E. japonica Leaves*

The enzyme was purified from 150 g leaves. Enzyme was assayed in 90 mM Na-phosphate (pH 8.0), 110 μM NADPH, and 10 mM DL-glyceraldehyde in a total volume of 1 ml at 25°C. One unit of activity is defined as the amount of enzyme that catalyzes the oxidation of 1 μmol NADPH/min.

| Purification Step	Volume	Protein	Total Protein	Specific Activity	Total Activity	Yield	Purification
	ml	*mg/ml*	*mg*	*units/mg*	*units*	*%*	*-fold*
Crude extract	558.0	7.18	4006.4	0.037	148.2	100	1.0
40–75% (NH₄)₂SO₄	48.5	50.59	2453.6	0.041	100.6	67.9	1.1
Sephacryl S-200	47.2	7.29	344.1	0.067	23.1	15.6	1.8
DEAE-Sephacel	22.5	3.98	89.6	0.325	29.1	19.6	8.8
Sephadex G-100	5.8	6.56	38.0	0.540	20.5	13.8	14.6
Reactive-Red 120-Agarose	3.7	1.02	3.8	5.240	19.8	13.3	141.6
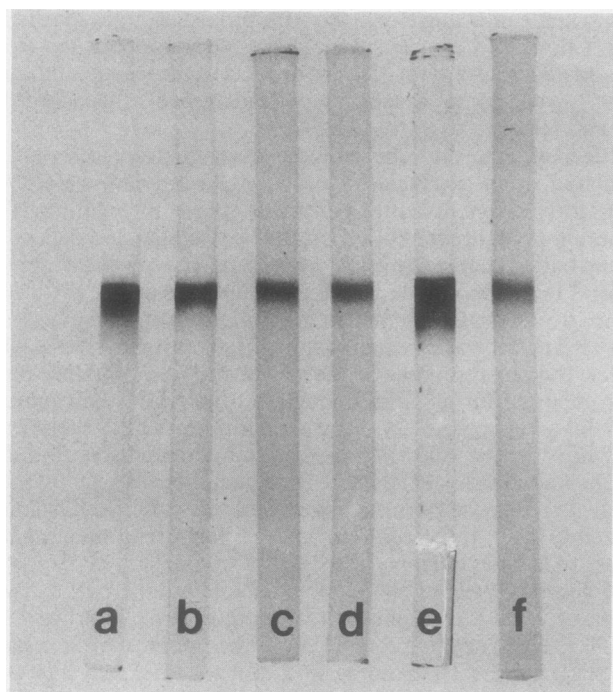
FIG. 1. Polyacrylamide disc gel electrophoresis of *E. japonica* aldose (aldehyde) reductase. Gels were stained for protein (a), or enzyme activity using propylene glycol (b), glycerol (c), galactitol (d), 3-pyridylcarbinol (e) for 20 min incubation period, and 3-pyridylcarbinol (f) for less than 5 min incubation period.
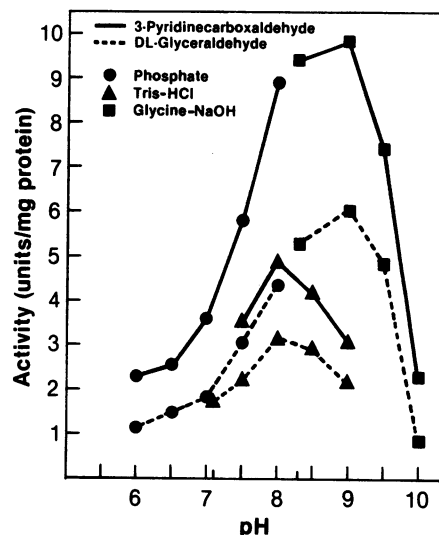

FIG. 2. Response of *E. japonica* aldose (aldehyde) reductase to the pH of the assay mixture using DL-glyceraldehyde or 3-pyridinecarboxaldehyde as a substrate. Assay mixture (1 ml) contained 90 mM buffer, 110 μM NADPH, affinity fraction, and 10 mM substrate to start the reaction.

the K_m for D-galactose which agreed with the fraction of the sugar in the free aldehyde form in solution. Recently, Inagaki *et al.* (17) provided evidence that bovine lens aldose reductase acted on the aldehyde form of D-glucose (K_m = 0.66 μM) rather than the α or the β anomers.

Results obtained with 2-deoxy sugars were notable. While D-glucose and D-ribose were inactive as substrates, their deoxy derivatives were reactive (Tables II and III). The K_m for 2-deoxy-galactose was 20 times less than that of D-galactose. The absence of the hydroxyl group at C_2 probably removes an unfavorable steric interaction with some groups on the enzyme. Conversely, the presence of an amino group at C_2 caused inactivity (*i.e.* galactosamine). Absence of the OH group at C_6 (D-fucose) or presence of COO⁻-group (D-galacturonic acid) reduced the reactivity of D-galactose. The *Euonymus* enzyme was 21% as active with L-fucose as with D-fucose, suggesting that the D-form of galactose is the preferred substrate.

Aliphatic aldehydes (C_2-C_6) were good substrates for the enzyme as compared to aldoses (Table II). The K_m value for these aldehydes decreased with increasing chain length. Similar results were obtained with rat liver and pig brain aldehyde reductases

(12, 29). Aromatic aldehydes were the best substrates (highest V_{max}/K_m ratio). Methyl- and phenylglyoxal derivatives showed higher activity than glyoxal, but pyridine substitution (*i.e.* pyridinecarboxaldehydes) resulted in a lower K_m value compared to phenylglyoxal.

Negligible rates were obtained in the reverse reaction with 3-pyridylcarbinol, ethanol, ethylene glycol, propylene glycol, L-arabitol, and xylitol at pH 9.5 using 90 mM glycine-NaOH and NADP (1 mM).

Phosphorylated sugars (glucose-6-P, galactose-6-P, 2-deoxyglucose-6-P, ribose-5-P, 2-deoxyribose-5-P) were not active substrates.

Product Inhibition. NADP was a competitive inhibitor when NADPH was the variable substrate and DL-glyceraldehyde concentration was kept constant at 10 mM. Using the Dixon plot (11), the K_i was 60 μM (Fig. 4). Glycerol appeared to be an uncompetitive inhibitor of DL-glyceraldehyde (Fig. 5). The K_i' value estimated from the Cornish-Bowden plot (7) was 460 mM. Studies with aldose and aldehyde reductases from mammalian sources showed that NADP was a competitive inhibitor relative to NADPH (9, 22, 25). Glycerol was a competitive inhibitor of DL-glyceraldehyde in one study (22) and noncompetitive inhibitor in others (9, 31).

Effect of Inhibitors. A number of chemicals were incubated with *Euonymus* reductase and NADPH for 5 min prior to substrate addition to test their inhibitory effect (Table IV). Pyrazol, a potent inhibitor of NAD-dependent alcohol dehydrogen-
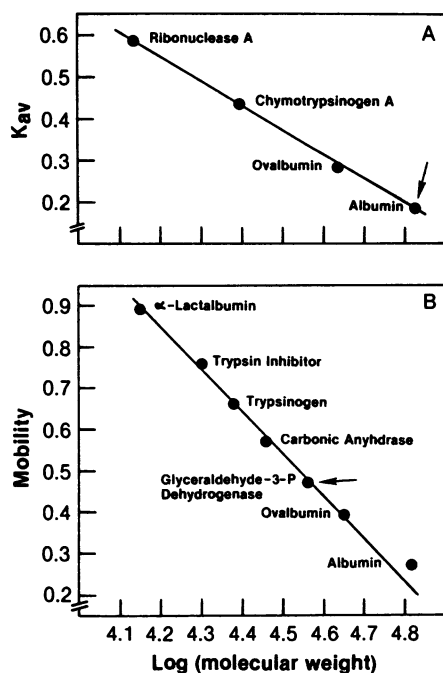
FIG. 3. Mol wt estimation of aldose (aldehyde) reductase from *E. japonica* by gel filtration on Sephadex G-100 (A) and by SDS gel electrophoresis (B). The arrow indicates the position of the enzyme in reference to standards.

Table II. *Substrate Specificity for Aldose (Aldehyde) Reductase from E. japonica Leaves*

K_m and V_{max} were calculated from Lineweaver-Burk plots using linear regression analysis. At least 10 different concentrations per substrate were used to generate these values. Reaction mixtures contained in 1 ml: 90 mM Na-phosphate (pH 8.0), 110 μM NADPH, affinity fraction, and substrate to start the reaction. The K_m value for NADPH was determined using 10 mM DL-glyceraldehyde as a substrate. Assays were carried out at 25°C.

Substrate	K_m	V_{max}
	mM	*units/mg protein*
D-Galactose	227.1	2.28
2-Deoxygalactose	10.6	5.21
L-Arabinose	287.2	1.81
L-Xylose	1174.3	4.78
2-Deoxyribose	17.5	4.75
D-Erythrose	28.1	5.41
DL-Glyceraldehyde	3.4	4.91
Glycoaldehyde	1.8	12.89
Glyoxal	18.9	1.81
Methylglyoxal	3.9	6.57
Phenylglyoxal	0.68	6.46
Benzaldehyde	0.295	9.51
p-Nitrobenzaldehyde	0.003	3.32
2-Pyridinecarboxaldehyde	0.099	6.71
3-Pyridinecarboxaldehyde	0.046	5.51
4-Pyridinecarboxaldehyde	0.004	3.30
Acetaldehyde	6.3	5.95
Propionaldehyde	5.6	6.18
Butyraldehyde	0.444	6.31
n-Valeraldehyde	0.087	4.21
Hexanal	0.034	6.42
NADPH	0.010	3.89

Table III. *Substrate Specificity of Aldose (Aldehyde) Reductase from E. japonica Leaves*

Reaction mixture contained in 1 ml: 90 mM Na-phosphate (pH 8.0), 110 μM NADPH, enzyme (affinity fraction), and substrate to start the reaction. Assays were conducted at 25°C.

| Substrate	Concentration	Activity
	mM	*units/mg*
D-Galactose	50	0.654
2-Deoxygalactose	50	4.810
D-Galactosamine	50	0.0
D-Galacturonic acid	50	0.188
D-Galactonic acid-γ-lactone	50	0.0
D-Fucose	50	0.341
L-Fucose	50	0.073
D-Glucose	50	0.0
D-Mannose	50	0.0
2-Deoxyglucose	50	0.459
D-Lyxose	50	0.109
D-Xylose	100	0.38
D-Xylose	10	0.042
D-Ribose	50	0.0
Dihydroxyacetone	50	0.073
Dihydroxyacetone	10	0.022
DL-Glyceraldehyde	10	5.045
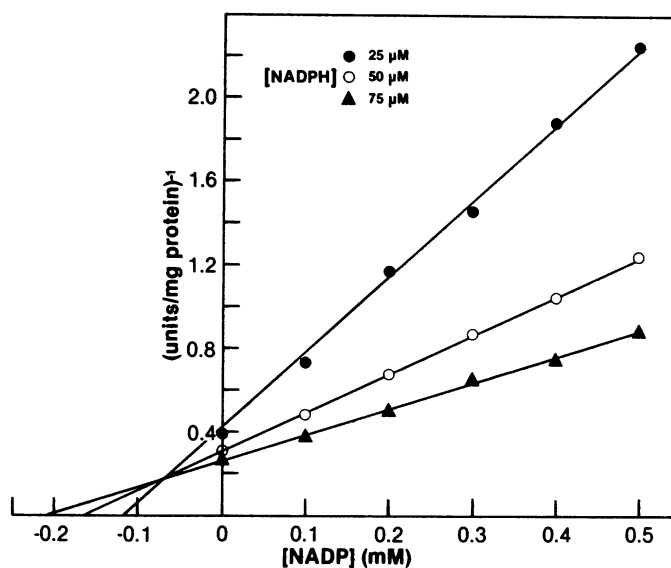
FIG. 4. Product inhibition of *E. japonica* aldose (aldehyde) reductase by NADP (Dixon plot). Assay mixture (1 ml) contained 90 mM Na-phosphate (pH 8.0), affinity fraction, NADP at the indicated concentrations, different levels of NADPH, and 10 mM DL-glyceraldehyde to initiate the reactions.

ase, caused slight inhibition. Metal chelating agents such as 2,2'-dipyridyl, and 1,10-phenanthroline at 10 mM caused 35 and 75% inhibition, respectively. EDTA at 5 mM caused 8% inhibition. On the basis of these data, the absence of a metal ion requirement cannot be ruled out.

Euonymus reductase was inhibited by p-hydroxymercuribenzoate, but iodoacetic acid, iodoacetadamide caused slight inhibition. These results clearly distinguish the *Euonymous* enzyme from the alcohol dehydrogenase. Alcohol dehydrogenases isolated from mammalian and plant sources reduce a wide range of aldehydes but are completely inhibited by pyrazol and metal chelating agents (2, 5). Phenobarbital was a strong inhibitor of *Euonymus* enzyme, similar to mammalian aldehyde reductase (12). Lithium sulfate was also inhibitory at 200 mM. However,
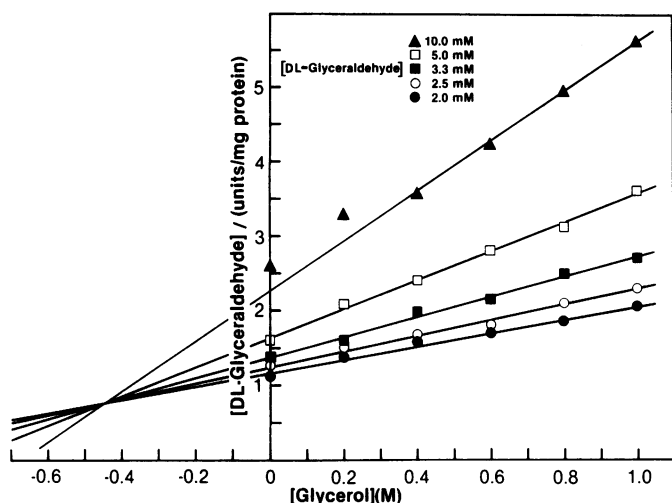
FIG. 5. Product inhibition of *E. japonica* aldose (aldehyde) reductase by glycerol (Cornish-Bowden plot). Assay mixture (1 ml) contained 90 mM Na-phosphate (pH 8.0), 110 μM NADPH, affinity fraction, glycerol at the indicated concentration, and DL-glyceraldehyde at different concentrations were used to initiate the reactions.

Table IV. *Effect of Inhibitors on E. japonica Aldose (Aldehyde) Reductase*

Reaction mixture contained in 1 ml: 90 mM Na-phosphate (pH 8.0), 110 μM NADPH, enzyme (affinity fraction), inhibitor, and 10 mM DL-glyceraldehyde to start the reaction. Enzyme, NADPH, and inhibitor were incubated for 5 min prior to substrate addition. Assays were conducted at 25°C.

Inhibitor	Concentration	Relative Activity
	mM	*%*
None		100
Pyrazol	1.0	89
Pyrazol	10.0	79
Pyrazol	20.0	65
Hydroxylamine	1.0	88
Hydroxylamine	5.0	62
Hydroxylamine	10.0	37
2,2'-Dipyridyl	1.0	94
2,2'-Dipyridyl	10.0	65
1,10-Phenanthroline	1.0	76
1,10-Phenanthroline	10.0	25
5,5-Diphenylhydantoin	0.5	97
5,5-Diphenylhydantoin	1.0	84
EDTA	5.0	92
Phenobarbital	1.0	20
Phenobarbital	10.0	3
Iodoacetamide	5.0	99
Iodoacetamide	10.0	74
Iodoacetic acid	5.0	95
Iodoacetic acid	10.0	83
p-Hydroxymercuribenzoate	1.0	0
N-Ethylmaleimide	5.0	71
Lithium sulfate	10.0	99
Lithium sulfate	200.0	56

activation by 400 mM Li_2SO_4 has been reported for aldose reductase from difference sources (8, 15).

Distribution of Aldose (Aldehyde) Reductase in the Celastraceae. Leaves and berries of different species of *Euonymus* were frozen and ground into fine powder in liquid N_2. Powders were extracted as described under "Materials and Methods" and $(NH_4)_2SO_4$ (40–75%) fractions were used for enzyme assays with

DL-glyceraldehyde and D-galactose as substrates as described previously. Enzyme activity was detected in the following *Euonymus* species: *E. atropurpurea*, *E. bungeana*, *E. europaea* 'Alba,' *E. europaea* 'Macrocarpa,' *E. europaea* 'Red Cascade,' *E. fortunei*, *E. hamiltoniana* var maackii, *E. hamiltoniana* var yedoensis 'Calocarpa,' *E. nana* var turkestanica, *E. obovata*, and *E. verrucosa*. The specific activity of the $(NH_4)_2SO_4$ fraction of the above species was between 0.06 to 0.16 units/mg protein using DL-glyceraldehyde as a substrate. The $(NH_4)_2SO_4$ fraction of leaves and berries of several cultivars of *E. alata*, however, did not show activity towards DL-glyceraldehyde or D-galactose even though the galactitol content of the leaves reaches 5 to 8% dry weight (data not shown). The reductase activity was also detected in *Celastrus orbiculatus* (specific activity of $[NH_4]_2SO_4$ fraction = 0.02 units/mg protein).

CONCLUSIONS

The results presented above showed a procedure for isolating the enzyme aldose (aldehyde) reductase from *Euonymus japonica* leaves with a high degree of purity. The enzyme was fairly stable at 1°C and can be stored at −65°C for more than 1 year.

Plant aldehyde reductases that were isolated from different sources showed a high degree of substrate specificity, *e.g.* fatty aldehyde reductase from *Brassica oleracea* (18) and indole-3-aldehyde reductase from *Cucumis sativus* (6). *Euonymus japonica* reductase, however, was capable of reducing aldoses and aliphatic and aromatic aldehydes. In view of the low reaction rate observed with alcohols, it appears that *Euonymus* enzyme functions in aldehyde reduction rather than alcohol oxidation. Since the physiological function of this enzyme is probably in the synthesis of galactitol from D-galactose, it is appropriate to suggest naming this enzyme aldose reductase (Alditol: NADP oxidoreductase).

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