Phytochemistry, Vol. 24, No. 4, pp. 689–693, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

MITOCHONDRIAL ALDEHYDE DEHYDROGENASE FROM HIGHER PLANTS

H. ASKER and D. D. DAVIES

School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ, U.K.

(*Revised received* 23 *August* 1984)

Key Word Index—*Solanum tuberosum*; Solanaceae; potato; *Pisum sativum*; Leguminosae; pea aldehyde dehydrogenase; mitochondria.

Abstract—Aldehyde dehydrogenase has been purified to homogeneity from mitochondria of potato tubers and pea epicotyls. Although the enzyme had a high affinity for glycolaldehyde it also had a high affinity for a number of other aliphatic and arylaldehydes. It is proposed that the codification glycolaldehyde dehydrogenase (EC 1.2.1.22) should be abandoned in favour of mitochondrial aldehyde dehydrogenase (EC 1.2.1.3). The purified enzyme showed esterase activity and had properties similar to those reported for the mammalian mitochondrial aldehyde dehydrogenase. Although the natural substrate(s) for the enzyme is not known, the kinetic properties of the enzyme are consistent with it playing a role in the oxidation of acetaldehyde, glycolaldehyde and indoleacetaldehyde.

INTRODUCTION

Aldehyde dehydrogenase is an enzyme which catalyses the oxidation of a number of aldehydes to the corresponding acids. Aldehyde dehydrogenases from mammals have been studied intensively [1] whereas there are only a few studies on aldehyde dehydrogenase from plants. The partial purification from pea seedling mitochondria of a NAD-linked dehydrogenase which oxidized glycolaldehyde to glycollate and acetaldehyde to acetate was reported [2]. Subsequently this enzyme, designated as glycolaldehyde dehydrogenase, was codified as EC 1.2.1.22. An acetaldehyde dehydrogenase, isolated from germinating leguminous seedlings [3] was shown to be present in the cytosol but no aldehyde dehydrogenase activity was detected in mitochondria isolated from peanut cotyledons [4]. An indoleacetaldehyde dehydrogenase has been demonstrated in mitochondria isolated from mung bean seedlings [5] and although this enzyme has been implicated in the biosynthesis of indoleacetic acid, little is known about the properties of this enzyme. Long chain aliphatic aldehyde dehydrogenases have been isolated from Jojoba seedlings [6] which are almost certainly distinct from the enzyme(s) which oxidize aromatic and short aliphatic chain aldehydes. However, since the mammalian dehydrogenases have a wide substrate specificity [1] it is possible that some of the catalytic activities reported from plants are associated with a single enzyme. In view of the importance of aldehyde oxidation in plants we report the kinetic properties and substrate specificity of mitochondrial aldehyde dehydrogenase isolated from potato tubers and pea seedlings.

RESULTS

Purification

A typical purification of aldehyde dehydrogenase from potato mitochondria is shown in Table 1. The pea enzyme showed the same pattern of purification. The selective extraction of the mitochondrial pellet with Triton removed most soluble proteins, including malic dehydrogenase. Final purification of the aldehyde dehydrogenase was achieved using 5'AMP-Sepharose which binds NAD^+-dependent dehydrogenases and ATP-dependent kinases. Both preparations were unstable during purification but when the purified enzyme was stored at 0° with sucrose (20%) and serum albumen (1%), there was very little loss of activity over 3–4 days.

Relative activity with aldehydes

Both enzyme preparations were examined for their dehydrogenase activity with a number of aldehydes. The results are shown in Table 2.

Table 1. Purification of mitochondrial aldehyde dehydrogenase from potato tubers

Step	Volume (ml)	Protein (mg/ml)	Enzyme activity (units/ml)	Specific activity (units/mg)	Purification fold	Yield (%)
Mitochondrial extraction	30	3.8	0.066	0.0174	—	100
Affinity chromatography	15	0.13	0.09	0.7	40.2	68

The results are based on 1 kg of potato tubers.

Table 2. Relative rates of oxidation of various aldehydes with mitochondrial aldehyde dehydrogenase isolated from potato tuber and pea seedlings

Substrate	Potato enzyme		Pea enzyme	
	Substrate concentration (μM)	Relative activities	Substrate concentration (μM)	Relative activities
Propionaldehyde	50	100	10	100
Glycolaldehyde	100	213	—	—
Acetaldehyde	25	100	10	90
Butyraldehyde	25	100	10	91
Cinnamaldehyde	25	60	25	84
Benzaldehyde	25	40	25	54
Pentaldehyde	12.5	44	10	34
Heptaldehyde	12.5	56	—	—
Hexanal	12.5	63	—	—
Formaldehyde	2000	75	2000	84
Glyoxal	?	19	?	11
Citral	—	—	100	15
Isopropionaldehyde	—	—	100	20
Indoleacetaldehyde	25	28	25	74

The results are expressed as relative activities, the activity with propionaldehyde as substrate is set at 100 as standard. (The relative activities were measured at pH 8.5.) Because of the instability of some of the aldehydes, stock solutions were sonicated immediately before use in the enzyme assays.

Effect of pH and inorganic phosphate on the activity of aldehyde dehydrogenase

We have examined the effect of pH and Pi on the activity of the potato enzyme with glycolaldehyde and indoleacetaldehyde as substrates as well as on the enzyme from peas with glycolaldehyde as substrate. The results are shown in Fig. 1.

Kinetic constants

(a) K_m *(apparent) for aldehydes at pH* 8.5. High concentrations of some aldehydes were inhibitory, but at concentrations below 100 μM the kinetics observed were Michaelian. The kinetic constants, determined from plots of $1/v$ vs. $1/s$, for a number of aldehydes are collected in Table 3.

(b) K_m *(apparent) for NAD at pH* 8.5. The kinetics observed when the NAD concentration was varied and the glycolaldehyde concentration was constant at 100 μM, were Michaelian. Plots of $1/v$ vs. $1/s$ gave K_m (NAD) = 73 μM for the potato enzyme and K_m (NAD) = 27 μM for the pea enzyme.

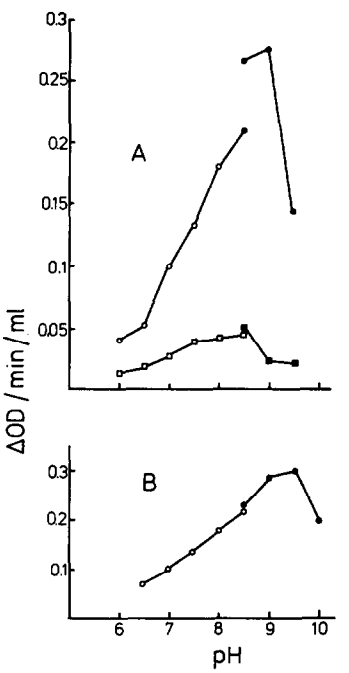

Fig. 1. Effect of pH on the activity of mitochondrial aldehyde dehydrogenase from potato tuber (A) and from pea seedlings (B) with glycolaldehyde and indoleacetaldehyde as substrates. Assays were carried out under standard conditions except that the pH was varied as indicated: ●—●, ○—○, Glycoaldehyde; ■—■, □—□, Indoleacetaldehyde; (○ □), Phospate buffer (0.1 M); (● ■), Glycine buffer (0.1 M).

Esterase activity of aldehyde dehydrogenase

Esterase activity was measured at pH 7, with *p*-nitrophenyl acetate as substrate in the presence and absence of NAD. The results are presented in Fig. 2. Esterase activity appears to be an inherent property of aldehyde dehydrogenases and reflects the intrinsic properties of the reactive amino acid residues at the active centre of the enzyme.

Table 3. Michaelis constants (K_m) and maximal velocities (V_{max}) for mitochondrial aldehyde dehydrogenases from potato tubers and pea seedlings with a number of aldehydes

Substrate	Potato enzyme		Pea enzyme	
	K_m (μM)	V_{max}	K_m (μM)	V_{max}
Glycolaldehyde	55	0.71	28	0.41
Acetaldehyde	—	—	1.6	0.25
Indoleacetaldehyde	27	0.14	25	0.2
DL-Glyceraldehyde	410	0.64	440	0.28

Kinetic constants were measured at pH = 8.5.

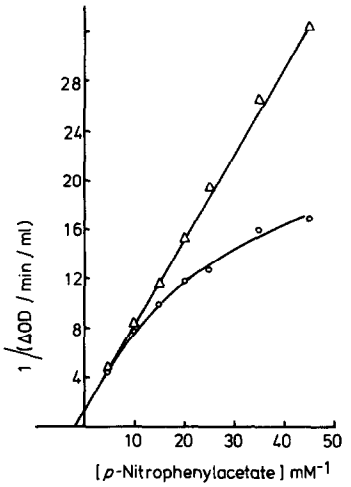

Fig. 2. Effect of varying the concentration of *p*-nitrophenylacetate on the esterase activity of the potato mitochondrial aldehyde dehydrogenase. Assays were carried out as described in the methods section in the presence (△—△) or absence (○—○) of NAD (1 mg/ml).

Substrate specificity

If a single enzyme is active with all the aldehydes, then the activity (V_t) with two aldehydes (S_1 and S_2) is given by the equation:

$$V_t = \frac{V_{max_1}[S_1]}{[S_1] + K_{m_1}\left(1 + \frac{[S_2]}{K_{m_2}}\right)} + \frac{V_{max_2}[S_2]}{[S_2] + K_{m_2}\left(1 + \frac{[S_1]}{K_{m_1}}\right)}.$$

If the observed activity with mixed substrates involves two specific dehydrogenases, then the activity (V_t) with two aldehydes is given by:

$$V_t = V_1 + V_2,$$

where $V_1 + V_2$ are the rates of reaction with substrates S_1 and S_2 respectively. The calculated and observed values with glycolaldehyde and acetaldehyde as substrates (Fig. 3) show poor agreement between the observed rate and that calculated assuming one *or* two enzymes. When glycolaldehyde and acetaldehyde were used together as substrates, there was a pronounced lag before the rate of reaction became linear. A similar effect was noted when

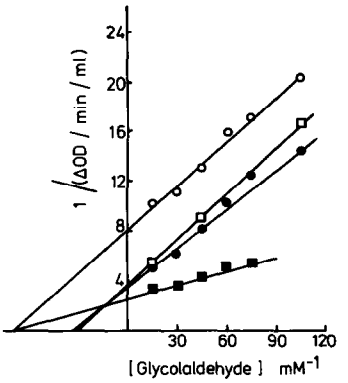

Fig. 3. Kinetic analysis of the oxidation of two aldehydes by purified aldehyde dehydrogenase from potato mitochondria. Activity was measured under standard conditions except that the substrates were varied as indicated: ●—●, measured activity with various concentrations of glycolaldehyde; ○—○, measured activity with various concentrations of glycolaldehyde *plus* a fixed concentration (70 μM) of indoleacetaldehyde; ■—■, calculated activity assuming 2 *enzymes* with both substrates present; □—□, calculated activity assuming a *single enzyme* is active with both substrates.

glycolaldehyde and cinnamaldehyde were used as substrates (Fig. 4). The observed steady state rate of aldehyde oxidation with two substrates present is compared in Table 4 with the rate calculated assuming two aldehyde dehydrogenases to be present.

Polyacrylamide gel electrophoresis

The aldehyde dehydrogenase activity present in crude extracts of mitochondria proved to be too unstable for polyacrylamide gel electrophoresis is (PAGE). The purified enzyme was sufficiently stable for PAGE and gave a single protein band which was active with a number of aldehydes.

DISCUSSION

After purification, the enzyme was relatively stable. However, during purification the enzyme was extremely unstable—mitochondrial extracts losing 50% of their activity in one hr at 0°. This instability may explain the failure to detect aldehyde dehydrogenase activity in peanut mitochondria [4].

PAGE established that a single protein band was active with a range of aldehydes and that only one band of activity could be demonstrated with the purified mitochondrial aldehyde dehydrogenase. The results of a kinetic analysis of aldehyde dehydrogenase with two substrates argue against the view that the purified preparation contains two enzymes. On the other hand the analysis did not produce clear-cut evidence for the view that a single enzyme was present.

The kinetics observed with indoleacetaldehyde and glycolaldehyde are somewhat unusual, but consistent with the suggestion that the preparation of indoleacetaldehyde contains an uncompetitive inhibitor. If this is the case, the true V_{max} with indoleacetaldehyde is greater than that measured.

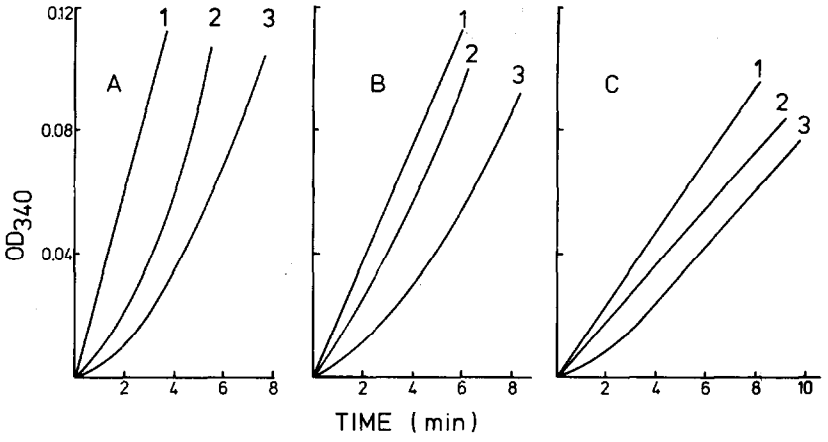

Fig. 4. Time course of product formation (NADH) with potato mitochondria aldehyde dehydrogenase in the presence of one substrate (glycolaldehyde) or two substrates (glycolaldehyde and acetaldehyde or glycolaldehyde with cinnamaldehyde). Assay conditions were standard except that the substrate composition and concentration were varied as indicated: A. 1 = glycolaldehyde alone (51.7 μM); 2 = mixed substrate glycolaldehyde (51.7 μM) + acetaldehyde (13.2 μM); 3 = mixed substrate glycolaldehyde (51.7 μM) + cinnamaldehyde (7.4 μM). B. 1 = glycoladehyde alone (12.9 μM); 2 = mixed substrate glycolaldehyde (12.9 μM) + acetaldehyde (13.2 μM); 3 = mixed substrate glycolaldehyde (12.9 μM) + cinnamaldehyde (7.4 μM). C. 1 = glycolaldehyde alone (7.4 μM); 2 = mixed substrate glycolaldehyde (7.4 μM) + acetaldehyde (13.2 μM); 3 = mixed substrate glycolaldehyde (7.4 μM) + cinnamaldehyde (7.4 μM).

Table 4. Kinetics of aldehyde oxidation catalysed by purified mitochondrial aldehyde dehydrogenase

Glycolaldehyde (Conc. μM)	Observed activity (ΔoD/min/ml) glycolaldehyde alone	A Observed activity (ΔoD/min/ml) glycolaldehyde + acetaldehyde (13.2 μM)	A[1] Calculated assuming 2 enzymes (both substrates present)	B Observed activity (ΔoD/min/ml) glycolaldehyde + cinnamaldehyde (10 μM)	B[1] Calculated assuming 2 enzymes (both substrates present)
60	0.3	0.28	0.44	0.3	0.39
30	0.22	0.24	0.36	0.22	0.31
20	0.19	0.19	0.33	0.18	0.28
15	0.15	0.15	0.29	0.15	0.24
12	0.12	0.13	0.26	0.11	0.21
8.6	0.09	0.12	0.23	0.1	0.18

Comparison between the *observed* rate of oxidation with various concentrations of glycolaldehyde in the presence of fixed concentrations of acetaldehyde (13.2 μM) or cinnamaldehyde (10 μM) and the *calculated* rates assuming 2 enzymes. The activity with acetaldehyde alone was 0.14 ΔoD/min/ml and with cinnamaldehyde alone was 0.09 ΔoD/min/ml. The activity was measured at pH 8.5 under standard conditions.

The kinetics observed with glycolaldehyde and either acetaldehyde or cinnamaldehyde showed a pronounced lag (Fig. 4). Although hysteresis has frequently been observed with mammalian aldehyde dehydrogenases, there is no satisfactory explanation of the lag. In the present context, the lag confounds the analysis based on the rate equation for mixed substrates. On the other hand the rates observed at the end of the lag phase (Table 4) are clearly not the sum of the individual rates with separate substrates and so argue against the presence of two enzymes.

It is not clear which aldehydes are the natural substrates of the enzyme. Mitochondria can catalyse the reactions of the arylpyruvate pathway for the synthesis of auxin [7] and the aldehyde dehydrogenase reported here may be the terminal enzyme of that sequence, but the high affinity for acetaldehyde and glycolaldehyde suggest that if they are formed in, or enter mitochondria, they will rapidly be oxidized to the corresponding acids.

The evidence suggests that the enzyme activities designated as glycolaldehyde dehydrogenase [2] and indoleacetaldehyde dehydrogenase [5] are manifestations of a single enzyme whose properties closely resemble those of the mammalian mitochondrial dehydrogenase. Thus a lag phase in the oxidation of aldehydes has been reported for the mammalian enzyme [8] and esterase activity appears to be an inherent property of the mammalian enzyme. However, it should be noted that

whereas NAD stimulates the esterase activity of the mammalian enzyme it inhibits that of the plant enzyme. It is therefore suggested that the codification of plant glycolaldehyde dehydrogenase as EC 1.2.1.22 should be changed to EC 1.2.1.3.

EXPERIMENTAL

Plant material. Pea seeds (*Pisum sativum* L. cv Meteor) were soaked overnight in aerated tap water. The seeds were then grown for 5 days in moist vermiculite at 25° in total darkness. Potato tubers (*Solanum tuberosum* L. cv Desiree) were purchased in a supermarket.

Chemicals. NAD, NADP, indole-3-acetaldehyde sodium bisulphite, glycolaldehyde and glyceraldehyde were purchased from Sigma, acetaldehyde, benzaldehyde and glyoxal sodium bisulphite were purchased from British Drug Houses and all other aldehydes were gifts from Dr. D. Land, Food Research Institute, Norwich. AMP-Sepharose was obtained from Pharmacia and all other chemicals were of the best grade commercially available.

Preparation of aldehydes. The bisulphite addition compound of indoleacetaldehyde was dissolved in a saturated soln of Na_2CO_3 and the pH adjusted to 10. After 5 min at room temp., the indoleacetaldehyde was isolated and assayed [10]. The bisulphite addition compound of glyoxal was similarly incubated with a soln of Na_2CO_3, but after 5 min, the soln was adjusted to pH 7, and either used directly or stored in ice for periods not exceeding 2 hr.

Preparation of aldehyde dehydrogenase from potato mitochondria. The enzyme in crude extracts was unstable and it was necessary to complete the following procedure in 10 hr. All operations were conducted at 0–2°. Peeled potato tubers (0.5 kg) were cut into 3 mm slices and vacuum infiltrated with an ice cold soln (A), containing Tris–HCl buffer (pH 8, 0.1 M), sucrose (0.5 M) and mercaptoethanol (2 mM). After air had been removed from the discs by applying a vacuum for 5 min, the vacuum was released. The infiltrated discs and fresh ice cold soln (A) were passed through a domestic Kenwood juice extractor, lined with Miracloth (Calbiochem) to retain cell wall debris and starch grains. The extract was centrifuged at 20 000 *g* for 20 min. The mitochondrial pellet was washed, resuspended in soln (A) and recentrifuged. The mitochondrial pellet was suspended in 250 ml MES buffer (pH 5, 25 mM) containing Triton (1%) and mercaptoethanol (5 mM) and stirred for 10 min. This suspension was centrifuged at 20 000 *g* for 20 min and the supernatant which was rich in malate dehydrogenase but lacking in aldehyde dehydrogenase was discarded. The pellet was washed again with the same soln and again the supernatant obtained by centrifugation at 20 000 *g* for 20 min was discarded. The pellet was suepended in 20 ml of K phosphate buffer (pH 7, 100 mM) containing Triton (1.5%) and mercaptoethanol (5 mM). After 10 min, the suspension was centrifuged at 30 000 *g* for 30 min to give a clear supernatant which contained some residual malate dehydrogenase and most of the aldehyde dehydrogenase activity, although an extra 10% of activity could be obtained by re-extracting the ppt. 20 ml of the clear supernatant was applied to an AMP-sepharose column (20 ml bed vol.) previously equilibrated with K phosphate buffer (pH 7, 50 mM) containing mercaptoethanol (5 mM) and the column was washed with 20 ml of the same soln. A further 10 ml of supernatant was applied to the column which was then washed until malic dehydrogenase could no longer be detected in the effluent. The enzyme was eluted from the column with K phosphate buffer (pH 7.5, 50 mM) containing NAD (10 mg/100 ml) and mercaptoethaNol (5 mM) and assayed in the effluent. The fractions containing aldehyde dehydrogenase activity were combined (16 ml) and sucrose (3 g) and serum albumin (16 mg) added to stabilize the enzyme.

Preparation of aldehyde dehydrogenase from pea mitochondria. Pea mitochondria were isolated from the epicotyls of 7 day old seedlings as described in ref. [11]. The enzyme was extracted from the mitochondria and purified as described above for potato mitochondria.

PAGE was effected by the method of ref. [12], except that electrophoresis was conducted with a constant current (2 mA/tube) and voltage (150 V). The run was stopped when the bromophenol blue had reached 1 cm from the bottom (3–4 hr). To detect enzyme activity gels were rimmed, transferred to culture tubes and covered with a staining soln containing glycolaldehyde (0.1 mM) or acetaldehyde (0.1 M) or DL-glyceraldehyde (0.5 mM), NAD (1.25 mM), *N*-methylphenazine methosulphate (0.1 mM), 3-(3,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (1 mM) and glycine buffer (0.1 M, pH 8.5). After incubation in the dark at 27° for 50 min, the gels were washed with tap water, then placed in 7.5% (v/v) HOAc and stored at 2°. Protein was stained with amido black.

Enzyme assays. Aldehyde dehydrogenase was assayed by measuring the increase in A_{340} associated with NAD oxidation. The assay mixture contained enzyme (50 μl), glycine buffer (0.75 mM) and glycolaldehyde (0.1 mM) in a total vol. of 1 ml. Assays were carried out at 25° in a spectrophotometer linked to a recorder. Because of non-enzymic reaction between NAD and glycolaldehyde, controls omitting the enzyme are essential.

Esterase activity was determined at 25° and at pH 7 with *p*-nitrophenyl acetate as substrate, by following the production of *p*-nitrophenol at 400 nm. Stock *p*-nitrophenyl acetate solns were made in Me_2CO, the final concn in the assay being less than 0.2%. Control experiments established that Me_2CO at this concn had no effect on the activity of the enzyme.

Alcohol dehydrogenase activity was assayed as described in ref. [13]. *Malic dehydrogenase* was assayed as described in ref. [14].

Units of enzyme activity. A unit of activity is defined as the formation of 1 μmol of NADH/min which is equivalent to an increase in A_{340} of 6.21/min. Sp. act. is defined as units of enzyme activity/mg of protein, determined by the method of ref. [15].

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