Phytochemistry, 1973, Vol. 12, pp. 523 to 530. Pergamon Press. Printed in England.

ALIPHATIC ALCOHOL DEHYDROGENASE FROM POTATO TUBERS

D. D. DAVIES, K. D. PATIL, E. N. UGOCHUKWU and G. H. N. TOWERS
School of Biological Sciences, University of East Anglia, Norwich NOR 88C

(*Revised Received* 20 *September* 1972. *Accepted* 5 *October* 1972)

Key Word Index—*Solanum tuberosum*; Solanaceae; potato; alcohol dehydrogenase; substrate specificity.

Abstract—Potato tubers are shown to contain at least 3 alcohol dehydrogenases, one active with NAD and aliphatic alcohols, one active with NADP and terpene alcohols and one active with NADP and aromatic alcohols. The purification of the aliphatic alcohol dehydrogenase is described and its activity with a wide range of substrates is reported. On the basis of substrate specificity, the enzyme is shown to resemble yeast alcohol dehydrogenase rather than liver alcohol dehydrogenase. The enzyme shows high activity with and high affinity for ethanol, activity and affinity decline as the chain length is increased from ethanol to butanol, but a further increase in chain length leads to increased affinity for the alcohol. The physiological significance of the results is briefly discussed.

INTRODUCTION

A NUMBER of studies have indicated that alcohol dehydrogenases from higher plants have broad substrate specificity. Cossins *et al.*[1,2] report that alcohol dehydrogenase from pea seedlings is active with primary, secondary and even tertiary aliphatic alcohols. Since tertiary alcohols do not possess an α-hydrogen atom which can be donated to NAD, it is difficult to see how a tertiary alcohol could act as a substrate. Duffus[3] reports that the enzyme from barley is active with a number of primary alcohols but inactive with secondary alcohols. Specificity for the cofactor also appears to vary from species to species thus pea alcohol dehydrogenase is specific for NAD[2,4] whilst the barley embryo alcohol dehydrogenase is active with either NAD or NADP.[3] A further problem of specificity relates to the oxidation of terpene alcohols. Thus liver alcohol dehydrogenase oxidises ethanol, geraniol and farnesol using NAD as a cofactor,[5,6] yeast alcohol dehydrogenase does not oxidize terpenols, whilst an enzyme from oranges, which oxidizes geraniol using NAD as a cofactor, is inactive with ethanol.[7] Extracts from peppermint catalyse the reduction of pulegone with NADPH rather than NADH.[8]

The stereospecificity of NAD and NADP reduction by various preparations of alcohol dehydrogenase have indicated that liver and yeast alcohol dehydrogenase transfer hydrogen from aliphatic and aromatic alcohols to the A side of the nicotinamide ring.[9–12] On the

[1] E. A. COSSINS and E. R. TURNER, *Ann. Bot.* **26**, 591 (1962).
[2] E. A. COSSINS, L. C. KOPALA, B. BLAWACKY and A. M. SPRONK, *Phytochem.* **7**, 1125 (1968).
[3] J. H. DUFFUS, *Phytochem.* **7**, 1135 (1968).
[4] C. E. ERIKSSON, *Acta Chem. Scand.* **21**, 304 (1967).
[5] G. R. WALLER, *Nature, Lond.* **207**, 1389 (1965).
[6] J. CHRISTOPHE and G. J. POPJAK, *Lipid Res.* **2**, 244 (1961).
[7] V. H. POTTY and J. H. BRUEMMER, *Phytochem.* **9**, 1003 (1970).
[8] J. BATTAILE, A. J. BURBOTT and W. D. LOOMIS, *Phytochem.* **7**, 1159 (1969).
[9] F. H. WESTHEIMER, H. F. FISHER, E. E. CONN and B. VENNESLAND, *J. Am. Chem. Soc.* **74**, 2402 (1951).
[10] H. F. FISHER, E. E. CONN, B. VENNESLAND and F. H. WESTHEIMER, *J. Biol. Chem.* **202**, 687 (1953).
[11] J. W. CORNFORTH, G. RYBACK, G. POPJAK, C. DONNINGER and G. SCHROEPFER, JR., *Biochem. Biophys. Res. Commun.* **9**, 371 (1962).
[12] K. DALZIEL and F. M. DICKINSON, *Biochem. J.* **95**, 311 (1965).

other hand enzymes have been isolated from liver[13] and from *Curvularia falcata*[14] which are active with aromatic substrates and in which hydrogen transfer is specific for the B side of NADP.

In this paper we report the separation of aliphatic and aromatic alcohol dehydrogenases from extracts of potato tubers and describe the properties of the aliphatic alcohol dehydrogenase with particular reference to substrate specificity and stereospecificity.

RESULTS

Purification and Properties of Potato Aliphatic Alcohol Dehydrogenases

The results of the purification procedure given in the experimental section are presented in Table 1. It can be seen that nearly 90-fold purification was acheived. During initial attempts to purify the enzyme, activity was rapidly lost on standing. Preparations were stabilized by including mercaptoethanol (5 mM) in all buffers and by including sucrose (2·5 g/10 ml) in the final preparation. Such preparations lost little activity over periods of 3–4 weeks.

TABLE 1. PURIFICATION OF ALIPHATIC ALCOHOL DEHYDROGENASE FROM POTATO TUBERS

Fraction	Volume (ml)	Protein (mg/ml)	Enzyme activity (units/ml)	Specific activity	Ratio Purification	Activity with acetaldehyde / Activity with benzaldehyde
Extract	1000	3·6	0·7	0·194	0	2·3
0–35% $(NH_4)_2SO_4$	50	22·6	0·7	0·031	—	2·0
35–50% $(NH_4)_2SO_4$	50	15·3	7·1	0·465	2·4	10·0
50–60% $(NH_4)_2SO_4$	50	4·3	1·7	0·394		5·3
35–50% $(NH_4)_2SO_4$ after dialysis for 16 hr	70	9·0	4·5	0·50	2·6	7·5
DEAE peak fraction (I)	4	0·035	0·6	17·2	88	83·0

Run 3. The units of activity and details of the purification are given in experimental.

During the purification, the activity of the fractions with various substrates was examined. Some of these results are shown in Table 1 and Fig. 1. The data from Table 1 indicates that separate enzymes are responsible for activity with aliphatic and aromatic aldehydes. The results in Fig. 1 suggest the presence of at least 3 alcohol dehydrogenases with overlapping specificity towards aliphatic and aromatic substrates.[15] The experiments reported in this paper are concerned with the properties of the aliphatic alcohol dehydrogenase represented by peak I (Fig. 1). Only the early fractions were combined to ensure that the enzyme was free from activity against aromatic alcohols.

Specificity of Aliphatic Alcohol Dehydrogenates for Nicotinamide Adenine Dinucleotides

The most highly purified fractions of the aliphatic alcohol dehydrogenase showed activity with NAD and NADP, though the activity with NADP was less than 5% of that observed with NAD. The results shown in Fig. 1 suggest the possibility that the activity

[13] H. W. CULP and R. E. MCMAHON, *J. Biol. Chem.* **243**, 848 (1968).
[14] V. PRELOG, *Pure Appl. Chem.* **9**, 119 (1964).
[15] D. D. DAVIES, E. N. UGOCHUKWU, K. D. PATIL and G. H. N. TOWERS, *Phytochem.* **12**, 53 (1973).

with NADP may represent contamination of the NAD specific enzyme with a NADP specific enzyme, which is eluted from the DEAE-cellulose, slightly ahead of the NAD aliphatic alcohol dehydrogenase. The kinetic constants for NAD in the presence of ethanol and butanol and for NADH in the presence of acetaldehyde and *n*-octanal are shown in Table 2.

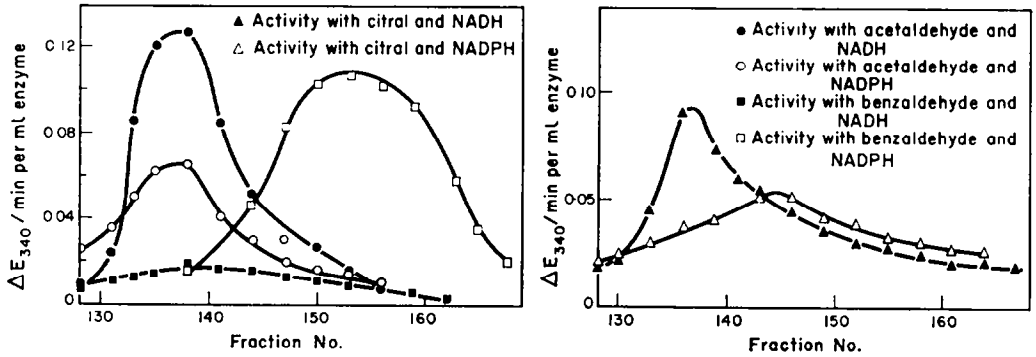

FIG. 1. SEPARATION OF ALCOHOL DEHYDROGENASE OF POTATO TUBERS BY ION EXCHANGE CHROMATOGRAPHY ON A COLUMN OF DEAE-CELLULOSE.
Run 5. For experimental details see the text. *Note.* The scale of activity with acetaldehyde + NADH is 10 × the scale for other activities.

Stereospecificity of Nicotinamide Adenine Dinucleotide Reduction by Potato Aliphatic Alcohol Dehydrogenase

The methods employed in the determination of stereospecificity were those described by Davies *et al.*[16] The potato enzyme was incubated with acetaldehyde and [^{3}H]-NADH, the tritium being present in the B position. When the reaction had reached completion, the NAD was isolated and its radioactivity measured. The results shown in Table 3 established that, like alcohol dehydrogenase from yeast, 10 liver, and wheat germ,[17] potato

TABLE 2. KINETIC CONSTANTS FOR NAD WITH ETHANOL AND BUTANOL AS SUBSTRATES AND FOR NADH WITH ACETALDEHYDE AND OCTANAL AS SUBSTRATES*

Nucleotide	pH of buffer	Constant substrate	K_m (apparent) (M × 10^4)	V_{max}	Nucleotide	pH of buffer	Constant substrate	K_m (apparent) (M × 10^4)	V_{max}
NAD	8·4	Ethanol (5 mM)	1·3	0·053	NADH	7·4	Acetaldehyde (6 mM)	2·8	0·57
NAD	8·4	*n*-Butanol (5 mM)	1·3	0·018	NADH	7·4	Octanal (0·5 mM)	1·2	0·021

* In Tris buffer 0·05 M.

[16] D. D. DAVIES, A. TEIXEIRA and P. KENWORTHY, *Biochem. J.* **127**, 335 (1972).
[17] H. R. LEVY and B. VENNESLAND, *J. Biol. Chem.* **228**, 85 (1957).

alcohol dehydrogenase is *A* specific. The purified enzyme also catalyses the reduction of cinnamaldehyde by NADH and the results shown in Table 3 demonstrage that with this substrate also the enzyme is *A* specific. This finding should be contrasted with the demonstration that the aromatic aldehyde reductase of rabbit kidney cortex[13] and the alcohol dehydrogenase from *Curvalaria falcata*,[14] both show specificity for the *B* side of the nicotinamide ring.

TABLE 3. STEREOSPECIFICITY OF NAD-LINKED ALIPHATIC ALCOHOL DEHYDROGENASE OF POTATO TUBERS

Substrate	Radioactivity in *B* position of [^{3}H] NADH (cpm)	Recovery of radioactivity in NAD (%)	Stereospecificity
Acetaldehyde	127 000	82	A
Cinnamaldehyde	625 000	76	A

For experimental details see the text.

Substrate Specificity of Aliphatic Alcohol Dehydrogenase

The relative insolubility of higher alcohols and particularly higher aldehydes necessitates the use of a solvent in enzyme assays but we found none that was entirely satisfactory. Methanol, which is a very poor substrate for alcohol dehydrogenase, has been used by some previous investigators[7] but, as expected, proved to be an inhibitor of the potato enzyme with *n*-pentanol or the aldehyde as substrates. The inhibition was complex, varying from apparent competitive to non-competitive and it is difficult to place confidence in kinetic constants determined in the presence of methanol. Tris buffers have been used by some previous investigators of alcohol dehydrogenase,[7,18–24] however, Tris is a trihydric alcohol and thus a potential substrate. Mahler[25] has reported that Tris is a substrate for liver alcohol dehydrogenase and competes with ethanol for active sites on the enzyme. Consequently we have examined the effect of Tris on the potato aliphatic alcohol dehydrogenase. Tris does not serve as a substrate nor does it inhibit the enzyme when either ethanol or propanol is used as substrate. However, Tris does react with aldehydes[26] and its use with substrates containing a reactive carbonyl group should be questioned. In addition to its use in the assay of alcohol dehydrogenase, Tris has been used in the assay of aldehyde reductase[27,28] and it appears to be a suitable buffer provided that the reaction is initiated by the addition of the aldehyde substrate. Kinetic constants for various aldehydes and ketones are presented in Table 4. The kinetic constants for various alcohols are presented in Table 5. A very wide range of other compounds has also been examined for activity with the enzyme. We draw

[18] K. B. JACOBSON, J. B. MURPHY, J. A. KNOPP and J. R. ORTIZ, *Arch. Biochem. Biophys.* **149**, 22 (1972).
[19] H. A. STAFFORD and B. VENNESLAND, *Arch. Biochem. Biophys.* **44**, 404 (1953).
[20] K. EBISUZAKI and E. S. G. BARRON, *Arch. Biochem. Biophys.* **69**, 555 (1957).
[21] J. VAN EYS and N. O. KAPLAN, *J. Am. Chem. Soc.* **79**, 2782 (1957).
[22] B. M. ANDERSON and N. O. KAPLAN, *J. Biol. Chem.* **234**, 1226 (1959).
[23] N. O. KAPLAN, H. M. CIOTTI and F. E. STOLZENBACH, *J. Biol. Chem.* **221**, 833 (1956).
[24] R. D. DE MOSS, *J. Bacteriol.* **68**, 252 (1954).
[25] H. R. MAHLER, *Ann. N.Y. Acad. Sci.* **92**, 426 (1961).
[26] P. F. DUGGAN, D. M. X. DONNELLY and D. P. MELODY, *Irish J. Med. Sci.* **460**, 163 (1964).
[27] S. BLACK, *Arch. Biochem. Biophys.* **34**, 86 (1951).
[28] R. M. BURTON and E. R. STADTMAN, *J. Biol. Chem.* **202**, 873 (1953).

attention to the following findings: (a) secondary alcohols are poor substrates; (b) activity with ketones is low—thus the activity with butane-2-one is only 0·5% of the activity with acetaldehyde; (c) the preparation is more active with allyl alcohol than with ethanol, but shows less activity with propargyl alcohol; and (d) *trans*-4-hydroxyproline acts as a substrate.

TABLE 4. KINETIC CONSTANTS OF POTATO ALCOHOL DEHYDROGENASE WITH VARIOUS ALDEHYDES AND KETONES

Aldehyde or ketone	K_m (mM)	V_{max} relative to acetaldehyde	Aldehyde or ketone	K_m (mM)	V_{max} relative to acetaldehyde
Tris at 0·25 M			Tris at 0·05 M		
Acetaldehyde	1·7	1·0	Formaldehyde	3·3	0·25
Propionaldehyde	2·5	0·6	Acetaldehyde	0·8	1·0
Butyraldehyde	10·0	0·12	Propionaldehyde	2·3	0·2
Pentaldehyde	1·6	0·52	Butyraldehyde	5·0	0·02
2-Methylbutyraldehyde	13·3	0·025	Pentaldehyde	2·0	0·44
Butan-2-one	21	0·033			

Conditions of assay: Tris buffer pH 7·4, 0·25 M or 0·05 M as indicated, NADH (0·4 mg) concentration of carbonyl compound varied and enzyme in a final volume of 3 ml.

Activity with Terpenoid Substrates

The purified preparation showed activity with geraniol, and citral. The activity with a saturated solution of citral was less than 4% of the activity observed with 5 mM acetaldehyde. The possibility that the fraction of aliphatic alcohol dehydrogenase used was contaminated with a terpene alcohol dehydrogenase cannot be excluded. The separation of activities towards various substrates shown in Fig. 1, indicates that an NADP terpene alcohol dehydrogenase is eluted between the peaks showing activity with aliphatic and aromatic alcohols.

TABLE 5. KINETIC CONSTANTS OF POTATO ALCOHOL DEHYDROGENASE WITH VARIOUS ALCOHOLS

Alcohol	K_m (mM)	V_{max} relative to rate with ethanol	Alcohol	K_m (mM)	V_{max} relative to rate with ethanol
Ethanol	7	100	Hexanol	6·0	33
Propanol	13·5	57	Heptanol	3·0	17
Butanol	18·8	34	Octanol	1·1	10
Pentanol	16·6	53	Isobutanol	30·3	—

Conditions of assay: Tris buffer pH 8·4, 0·05 M, NAD (0·4 mg) concentration of alcohol varied and enzyme in a final volume of 3 ml.

DISCUSSION

The results indicate that potatoes contain at least three alcohol dehydrogenases—an aromatic alcohol dehydrogenase which appears to be relatively specific for NADP, a terpene alcohol dehydrogenase which has greater activity with NADP than with NAD and an aliphatic alcohol dehydrogenase which is relatively specific for NAD.

The aliphatic alcohol dehydrogenase demonstrates the broad substrate specificity characteristic of both yeast and liver alcohol dehydrogenase.[29] However, it is difficult to be certain that the very low levels of activity observed when certain substrates are used at high concentrations, reflect the true substrate specificity of the enzyme. When substrates are tested at very high concentrations, impurities may reach significant concentrations in the assay mixture and produce erroneous results. An impurity present in tertiary amyl alcohol could explain the finding that tertiary amyl alcohol is a substrate for pea alcohol dehydrogenase.[2] Another difficulty arises from the uncertain purity of the enzyme preparation which may contain more than one alcohol dehydrogenase. These considerations complicate the interpretation of data on substrate specificity and obscure the relationship between alcohol dehydrogenase from yeast, liver and potatoes. A comparison of the three enzymes with respect to substrate specificity is shown in Table 6. However, attention is particularly drawn to the following considerations which suggest that the potato enzyme resembles yeast alcohol dehydrogenase rather than liver alcohol dehydrogenase: (a) methanol is a substrate; (b) activity with ethanol is greater than with butanol; (c) activity with acetaldehyde is greater than with benzaldehyde; (d) activity with acetaldehyde is greater than with *N*-butyraldehyde; and (e) cyclohexanol is not a substrate.

TABLE 6. COMPARISON OF SUBSTRATE SPECIFICITY OF VARIOUS ALCOHOL DEHYDROGENASES

Enzyme	Yeast alcohol dehydrogenase					Liver alcohol dehydrogenase					Higher plant alcohol dehydrogenase			
								Horse		Rat	Pea		Potato	
Substrate conc.	∞	0·3 M	0·2 M	0·1 M	5 mM	∞	1 mM	1 mM	∞	0·54 M	10 mM	50 mM	5 mM	0·1 M
pH	9·3	8·4	8·9	8·2	9·3	7·0	9·5	9·5	7·0	8·8	8·2	8·2	8·4	8·4
Reference	22	30	31	20	21	32	33	34	32	35	4	2	36	36
Substrate														
Methanol	4						0					0·4	0	1
Ethanol	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Propanol		44	36	38	65		110	36	120	160	18	36	42	
Propan-2-ol	39	27	7	1·6					10	47		0·†		2
Butanol		26	18	36	60		163	120	108	194	27		55	
Isobutanol		17			0·4				108	106				10
Butan-2-ol				0·4			36	36	22					4
Pentanol		14					118	33					16	
Pentan-3-ol						0			35					1·5
Allyl alcohol			100				142				141		167	
Cyclohexanol						0	100					0		0·2

All values have been calculated as a percentage of the rate with ethanol as substrate.

These points are not absolute—thus whilst it is generally stated that liver alcohol dehydrogenase is inactive with methanol, formaldehyde is a substrate. The statement that cyclohexanol is *not* a substrate for potato alcohol dehydrogenase is too strong, in fact activity with cyclohexanol was just detectable. This qualified statement should be balanced against the observation that the liver enzyme shows approximately as much activity with cyclohexanol as with ethanol.

Attention is drawn to the following considerations which suggest, that the potato

[29] H. SUND and H. THEORELL, *The Enzymes* (edited by P. D. BOYER, H. LARDY and K. MYRBACK), Vol. 7, p. 25, Academic Press, New York (1963).
[30] T. KELETI, *Arch. Physiol. Acad. Sci. Hung.* **13**, 103 (1958).
[31] E. S. G. BARRON and S. LEVINE, *Arch. Biochem. Biophys.* **41**, 175 (1952).
[32] F. M. DICKINSON and K. DALZIEL, *Biochem. J.* **104**, 165 (1967).
[33] A. D. WINER, *Acta. Chem. Scand.* **12**, 1695 (1958).
[34] A. D. MERRITT and G. M. TOMKINS, *J. Biol. Chem.* **234**, 2778 (1959).
[35] M. J. ARSLANIAN, E. PASCOE and J. G. RHEINGOLD, *Biochem. J.* **125**, 1039 (1971).
[36] This paper.

enzyme resembles liver alcohol dehydrogenase rather than yeast alcohol dehydrogenase: (a) pentan-3-ol is a substrate; and (b) the preparation is active with terpene alcohols and aldehydes. However the activity with pentan-3-ol is less than 2% of the activity with ethanol and the presence of a minor impurity in the pentan-3-ol cannot be ruled out. It should be noted that in the case of the liver enzyme V_{max} with pentan-3-ol is approximately one-third the V_{max} with ethanol. The activity with terpene alcohols could be due to the presence of a specific terpene alcohol dehydrogenase.

On balance it appears that the substrate specificity of the potato enzyme is similar to that of yeast alcohol dehydrogenase. Isoenzymes of alcohol dehydrogenase have been reported in yeast,[37,38] liver[39] and higher plants.[40–42] The properties of potato alcohol dehydrogenase reported in this paper are presumably those of a population of isoenzymes. The comparison with alcohol dehydrogenases from various sources is valid since these also represent populations of isoenzymes. Difficulties of interpretation, however, become apparent when the physiological significance of the enzyme is considered.

Current opinion on liver alcohol dehydrogenase is that its role is to remove ethanol from the blood.[43] The physiological role of alcohol dehydrogenase in plants is generally taken to be the reduction of acetaldehyde formed under anaerobic conditions. However, in the case of potato tubers, pyruvic decarboxylase is absent or very low in activity.[44] Acetaldehyde could be formed by a number of alternative routes, e.g. from threonine,[45] deoxyribose phosphate[46] or β-alanine[47] but under anaerobic conditions potato tubers produce lactic acid rather than ethanol.[48] Thus the role of the enzyme in potato tubers is uncertain and its involvement in the metabolism of higher alcohols (e.g. hexanol or leaf alcohol) should be considered.

After prolonged periods of anaerobiosis, potato tubers produce ethanol and on subsequent transfer to air the ethanol disappears.[48] The simplest assumption is that a single alcohol dehydrogenase is involved in the formation and removal of ethanol. However the proposal[37,38] that the isoenzymes of yeast alcohol dehydrogenase are specialized—one functioning in the production of ethanol, the other in ethanol utilization—suggests that the alcohol dehydrogenase of potato tubers should be isolated after prolonged anaerobiosis and compared with the enzyme reported in this paper.

Finally, we draw attention to the kinetic constants for alcohols and aldehydes in relation to chain length. The affinity of the enyme for alcohols decreases as the chain length increases from C_2 to C_4 but then the affinity increases with increasing chain length (Table 5). A similar situation occurs with the aldehydes—the affinity for aldehydes decreases as the chain length increases for C_2 to C_4 but the affinity for pentaldehyde is significantly greater than for butyraldehyde (Table 4).

[37] U. Lutstorf and R. Megnet, *Arch. Biochem. Biophys.* **126**, 933 (1968).
[38] L. Schimpfessel, *Biochim. Biophys. Acta* **151**, 317 (1968).
[39] J. P. von Wartburg, U. M. Bretz-Lutstorf and T. M. Schenker, *8th Int. Cong. Biochem.* p. 87 (1970).
[40] G. E. Hart, *Molec. Gen. Genet.* **3**, 61 (1971).
[41] Y. Efron, *Science* **170**, 751 (1970).
[42] D. Schwartz, *Science* **164**, 585 (1969).
[43] H. A. Krebs and J. R. Perkins, *Biochem. J.* **118**, 635 (1970).
[44] W. O. James, *Adv. Enzymol.* **18**, 281 (1957).
[45] D. M. Greenberg, in *Metabolic Pathways* (edited by D. M. Greenberg), Vol. 2, p. 92, Academic Press, New York (1961).
[46] E. Racker, *J. Biol. Chem.* **196**, 347 (1952).
[47] A. Pihl and P. Fritzson, *J. Biol. Chem.* **215**, 345 (1955).
[48] D. F. Forward, in *Plant Physiology* (edited by F. C. Steward), Vol. IVA, Academic Press, New York (1965).

EXPERIMENTAL

Chemicals. NAD, NADP, NADH and NADPH were obtained from Boehringer Corp. (Lond.) Ltd., London W5. Trizma base and hydroxyproline were obtained from Sigma (London) Chemical Co., London SW6. The higher alcohols and most of the aldehydes and ketones were gifts from Dr. Derek Lund, Food Research Institute, Colney Lane, Norwich. Compounds were distilled under N_2. DEAE-cellulose was obtained from Whatman. Other chemicals were obtained from British Drug Houses, Poole, Dorset, U.K.

Enzyme preparation. Potato tubers were peeled and (750 g) passed through a domestic Kenwood centrifuge juice extractor together with 500 ml of Tris buffer (0·1 M, pH 7·4, 5 mM mercaptoethanol). The juice extractor was lined with 'Miracloth' obtained from Calbiochem., to yield a clear potato extract (750 ml). $(NH_4)_2SO_4$ (184 g) was added to the extract and after stirring for 10 min, the precipitated protein was removed by centrifugation at 13 000 *g* for 10 min. $(NH_4)_2SO_4$ (79 g) was added to the supernatant and after stirring for 10 min the precipitated protein was collected by centrifuging at 13 000 *g* for 10 min. The precipitate was dissolved in 50 ml of Tris buffer (pH 8·4, 0·05 M) containing 5 mM mercaptoethanol. The supernatant was treated with $(NH_4)_2SO_4$ (52 g) and after stirring for 15 min the precipitated protein collected by centrifuging at 13 000 *g* for 10 min was dissolved as described above. Activity was usually concentrated in the middle fraction but if significant activity was found in the third fraction, the 2 fractions were combined. The fraction was dialysed overnight against 5 l. of Tris buffer (0·05 M, pH 8·4) containing 0·05 M mercaptoethanol. The extract was clarified by centrifuging at 13 000 *g* for 15 min and applied to a column (42 × 2·5 cm) packed with DEAE-cellulose previously equilibrated with Tris buffer (pH 8·4, 0·05 M) containing mercaptoethanol (5 mM). The column was eluted by applying a linear concentration gradient obtained by placing 250 ml Tris buffer (pH 8·4, 0·05 M) in the mixing cylinder and an equal volume of Tris buffer (pH 7·4, 0·25 M) in the reservoir. Fractions (45 drops) were collected and assayed for activity. Active fractions were preserved by adding sucrose (2·5 g/10 ml). All steps were carried out at 2°.

Enzyme assays. The standard assay was carried out at pH 7·4 by measuring the decrease in extinction at 340 nm associated with NADH oxidation. The assay mixture contained Tris buffer (pH 7·4, 0·05 M) NADH (0·4 mg) and enzyme (0·1 ml) in a vol. of 3 ml. The reaction was started by the addition of 0·1 ml acetaldehyde (3×10^{-2} M). Under these conditions, the relation between reaction rate and enzyme concentration was linear. Assays were carried out at 30° with a Unicam SP 500 spectrophotometer.

Unit of enzyme activity. A unit of enzyme producing an ΔE_{340} of 1·0/min. Specific activity is defined as the number of units of enzyme activity/mg of protein. Protein was measured by the method of Warburg and Christian.[49]

Acknowledgements—E.N.U. and K.D.P. wish to thank the Association of Commonwealth Universities for award of a Staff Fellowship and Scholarship respectively, and E.N.U. also wishes to thank the University of Nigeria, Nsukka, for study leave.

[49] O. WARBURG and W. CHRISTIAN, *Biochem. Z.* **310**, 384 (1942).