Phytochemistry, 1976, Vol. 15, pp. 813–815. Pergamon Press. Printed in England.

SHORT REPORTS

SOYBEAN ALCOHOL DEHYDROGENASE

SYLVA LEBLOVÁ and EVA PERGLEROVA
Biochemistry Department of Natural Science Faculty, Charles University, Prague 2, Czechoslovakia

(*Revised received* 22 *July* 1975)

Key Word Index—*Glycine max*; Leguminosae; soybean; alcohol dehydrogenase.

Abstract—Alcohol dehydrogenase was prepared from germinating soybean seeds. Specific activity was increased from 511 to 31316 units. The coenzyme is NAD with a K_m of 10^{-4}M. Allyl alcohol is oxidized faster than ethanol; with the latter substrate, the K_m is 1.3×10^{-2}M, and the pH optimum 8.7. The enzyme catalyses acetaldehyde reduction, with a K_m of 10^{-2}M and a pH opt of 7.1. The MW is $53(\pm 5) \times 10^{-3}$.

In plants pyruvate can be converted to acetaldehyde, ethanol being formed during reoxidation of NADH. EtOH is actually formed during germination in amount *ca* 30 times more than lactate. Alcohol dehydrogenase reaches a maximum activity during swelling before the maximum of ethanol conentration [1].

Alcohol dehydrogenase (ADH) was isolated from germinating soybean seeds by precipitation of Na–Pi extract with $(NH_4)_2SO_4$ at 35–50% saturation, the active sediment being desalted on a column of Sephadex G-25 and separated on DEAE-cellulose. Using this procedure, activity of the enzyme preparation was increased from 511 to 31316 units per mg protein (Table 1).

The MW of soybean ADH is $53(\pm 5) \times 10^{-3}$. SH groups and metal are essential for activity as the enzyme is inhibited by NaN_3, phenanthroline, salicylaldoxime, cupral and iodacetamide (Table 2). Preincubation of the enzyme with the inhibitor enhances the inhibitive effect, while the binary complex enzyme-NAD weakens the inhibitory effect of NaN_3, salicylaldoxime and cupral. A similar effect is obtained from the enzyme-substrate complex in the case of salicylaldoxime and NaN_3.

Table 3 shows the inhibitory effect of oximes and amides on the oxidation rate of soybean ADH. The type of inhibition was studied by the Lineweaver–Burk method. The enzyme is inhibited by acetamide, butyryla-

Table 1. Purification procedure for soybean alcohol dehydrogenase

Treatment	Total protein (mg)	Total act. (units × 10^3)	Spec. act (units/mg protein)
Crude extract	2830	1450	511
35–50% $(NH_4)_2SO_4$ fraction	297	774	2610
Desalted $(NH_4)_2SO_4$ fraction	226	699	3090
DEAE column chromatography	15	470	31316

Table 2. Effect of chelators and substances binding -SH groups on activity of soybean ADH

Inhibitor	Inhibitor concn. (M)	Percentage inhibition			
		1	2	3	4
NaN_3	0.001	63	67	54	53
o-Phenanthroline	0.001	71	86	69	70
Salicylaldoxime	0.0005	47	83	31	26
Cupral	0.04	64	78	45	67
Iodacetamide	0.004	32	52	33	30

1-No preincubation
2-enzyme incubated for 5 min. with inhibitor
3-enzyme incubated for 5 min. with enzyme } before addition of inhibitor.
4-enzyme incubated for 5 min. with ethanol }

Table 3. Effect of amides and oximes on oxidation rate of soybean ADH (EtOH concn. 0.1M)

Inhibitor	Inhibitor Concn. (M)	Inhibition percentage			
		1	2	3	4
Acetoxime	0.01	54	73	14	53
Cyclohexanoneoxime	0.01	51	74	56	56
Acetamide	0.2	48	60	30	48
Butyrylamide	0.1	83	87	80	82

1-No preincubation
2-enzyme preincubated for 5 min. with inhibitor
3-enzyme preincubated for 5 min. with NAD
4-enzyme preincubated for 5 min. with ethanol
} before addition of inhibitor.

mide and cyclohexanoneoxime non-competitively and by acetoxime competitively with respect to the substrate. The binary complex enzyme–NAD weakens the inhibitory effect of acetoxime and acetamide.

Acetate, malate, lactate, succinate and pyruvate are all non-competitive inhibitors of soybean ADH. The inhibition constants are of the order of 10^{-2}M. These substances present *in vivo*, were tested as possible modulators and some of them as possible hydrogen acceptors for the enzyme–NADH complex, since the rate of reoxidation of this complex is the limiting step of the rate of EtOH oxidation. The effect of these substances is compared with their effect on the enzyme of rat liver [2] in Table 4.

Substrate specificity is demonstrated in Table 5. Among the alcohols tested, methanol, *n*-pentanol, isopropanol, isooctanol, 1,3-butanediol, 1,4-butanediol, cyclohexanol, glycerol, benzyl alcohol, 2-mercaptoethanol, 2-aminoethanol, sugar alcohols and terpenic alcohols remained unattacked.

The K_m values of soybean ADH is 1.3×10^{-2}M for ethanol, 1.00×10^{-2}M for allyl alcohol oxidation and 1.0×10^{-3}M for acetaldehyde reduction. The soybean ADH catalyses the oxidation of alcohols with a pH optimum of 8.7 and the reduction of acetaldehyde at pH optimum of 7.1. The coenzyme of this enzyme is NAD, the K_m value of which is 10^{-4}M; with NADP the oxidation of substrate is 100 times slower.

It can be concluded that soybean ADH is similar to the enzyme from human, horse and rat liver (LADH), which detoxified ethanol which is a unnatural product for the human organism, and to the yeast enzyme (YADH). The similarity with LADH lies in the MW (rat ADH 65000; YADH 150000) [2, 3], with LADH and YADH that SH groups are present in the catalytic centre and a metal participates in the reaction/Zn with LADH and YADH [4–7]. A similar mechanism for the catalysis with LADH is indicated by the qualitatively equal inhibition by oximes and amides (however the K_i values are 100 higher than for LADH [8–10]), but the effect of carbohydrate metabolites is not equal with LADH (Table 4). The Michaelis constants for EtOH and for NAD resemble those of YADH [3]. Soybean ADH oxidizes a number of monovalent alcohols at a rate which decreases with increasing chain length. Similar behaviour is observed with YADH. The soybean ADH oxidized allyl alcohol more rapidly than the saturated analogue like horse LADH; however soybean ADH does not attack cyclohexanol and *sec*-butanol, unlike horse LADH.

EXPERIMENTAL

Experimental material consisted of soy seeds, *Glycine max*, germinating for 40 hr; 20 g seeds were moistened with 60 ml H_2O in Petri dishes 19 cm in diam. Germination took place in thermostats at 18°.

Enzyme isolation. Alcohol dehydrogenase was isolated from 80 g germinated seeds which were homogenised with 80 ml cool 0.1 M Na-Pi buffer pH 8.5. Here as well as in all other solns employed, 0.01M mercaptoethanol was added. The homogenate was centrifuged for 20 min. at 20000 *g* at low temp. and the supernatant obtained was made up to 100 ml with Na–Pi buffer and further treated. The next step in purification was precipitation of the phosphate extract with $(NH_4)_2SO_4$ to 35% and then precipitation of the supernatant

Table 4. Relative oxidation rate of 0.1 M EtOH by ADH in presence of sugar metabolism intermediates at 0.1 M concn.

Intermediate	Oxidation rate	
	Soybean ADH	Rat ADH[2]
None	100	100
Lactate	62	99
Pyruvate	68	0
Acetate	21	100
Malate	33	0
Succinate	51	125
Isocitrate	97	not measured

Table 5. Substrate specificity of ADH

Alcohol	Alcohol concn. (M)	Relative Rate of Oxidation
EtOH	0.01	100
n-PrOH	0.01	48
2-Propen-1-ol	0.01	155
n-BuOH	0.01	40
2-Butane-1-ol	0.01	34
Isoamyl alcohol	0.01	12
4-Pentene-1-ol	0.01	6
n-Hexanol	satd	11
Cinamyl alcohol	satd	12
2-Phenylethanol	satd	7

to 50% saturation. This fraction was desalted on Sephadex G-25. The desalted sample containing roughly 100 mg protein was then purified by means of chromatography on DEAE-cellulose. A column, 2.4 × 45 cm in size was eluted with Tris–acetate buffer pH 6.4 with a linear gradient from 0.1 to 0.6M, using a vol of 1000 ml. Fractions of 12 ml were taken. All steps in enzyme purification were carried out in an ice box. The active fractions obtained were concentrated by means of lyophylisation; activity of the lyophilised preparations was maintained for several weeks.

Enzyme activity measurement. We determined the activity of the alcohol dehydrogenase by a procedure similar to that of Racker's method [11]. We determined proteins by the Lowry method [12]. The MW was determined by means of gel filtration on a column of Sephadex G-200, 1.6 × 18 cm in size. Elution was done with 0.01M Tris–acetate buffer pH 6.4 at a flow-rate of 3 ml/20 min.

REFERENCES

1. Leblová, S., Vaníčková, V. and Sinecká, E. (1974) *Biol. Plant.* **16,** 406.
2. Arslanian, M. J., Pascoe, E. and Reihold, J. G. (1971) *Biochem. J.* **125,** 1039.
3. Hayes, J. E. and Velick, S. F. (1954) *J. Biol. Chem.* **207,** 225.
4. Shore, J. D., and Theorell, H. (1967) *European J. Biochem.* **2,** 32.
5. Reynolds, C. H., Morris, D. L. and McKinley-McKee, J. S. (1970) *European J. Biochem.* **14,** 14.
6. Theorell, H. and McKinley-McKee, J. S. (1961) *Acta Chem. Scand.* **15,** 1811.
7. Reynolds, C. H. and McKinley-McKee, J. S. (1975) *Arch. Biochem. Biophys.* **168,** 145.
8. Nachman, M., Lester, D. and LeMagnen, J. (1970) *Science* **168,** 1244.
9. Lester, D. and Benson, G. D. (1970) *Science* **169,** 282.
10. Theorell, H. and McKinley-McKee, J. S. (1961) *Acta Chem. Scand.* **15,** 1834.
11. Racker, E. (1950) *J. Biol. Chem.* **184,** 313.
12. Lowry, H. O., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265.

Phytochemistry, 1976, Vol. 15, pp. 815–816 Pergamon Press. Printed in England.

THE *N*-TERMINAL AMINO ACID SEQUENCE OF CYTOCHROME *f* FROM *SPIRULINA PLATENSIS*

S. SANDERSON, B. G. HASLETT and D. BOULTER

Department of Botany, University of Durham, Durham DH1 3LE, England

(*Received* 8 *August* 1975)

Key Word Index—*Spirulina platensis*; amino acid sequence; cytochrome *f*; blue–green algae.

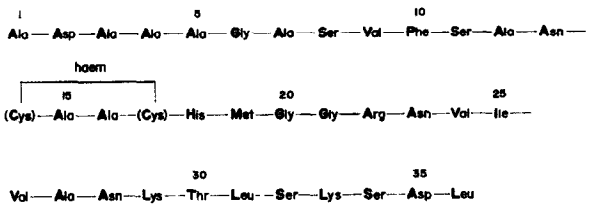
Ambler *et al.* [1] have recently reported the amino acid sequence of cytochrome *f* from the blue–green alga, *Spirulina maxima*, comparing it with other known cytochrome *f* sequences. We had at that time begun a study of this protein from a related species, *S. platensis*, from which we obtained a partial sequence.

Our data suggest a moderate degree of divergence between the proteins from the two species, since they differ at two residues out of the first 36 of the *N*-terminal region, namely at residue positions 1 and 3 (Fig. 1), where Gly and Val in *S. maxima* are replaced by alanine.

Studies on ferredoxin sequences [2] and DNA base compositions [3] also support the suggestion that morphological divergence has not kept pace with DNA and protein evolution in the blue–green algae. As a consequence, a relatively large number of sequences will need to be determined before the phylogenetic relationships between this and other groups of organisms can be established.

EXPERIMENTAL

A gift of partially purified cytochrome *f*, separated by an ammonium sulphate precipitation (55–80%



Residues 1–13, 15 and 16, and 19–36 were obtained by the sequencer method; residue 19 (His) has not been unambiguously identified. Residues 1–4 were confirmed by the manual method [5].

Fig. 1. *N*-terminal amino acid sequence. Sample 17 contained haem, thus allowing the positioning of Cys 14 and 17.