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THE AMINO ACID COMPOSITION OF SOYA LACTATE DEHYDROGENASE

JANA BARTHOVÁ, M. SEMANSKÝ and SYLVA LEBLOVÁ

Department of Biochemistry, Charles University, Albertov 2030, 128 40 Prague 2, Czechoslovakia

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Abstract—Lactate dehydrogenase from germinating soya plants was prepared in an electrophoretically homogeneous form by affinity chromatography on AMP–Sepharose. The isoelectric point of this enzyme determined by isoelectric focusing was 7.0. The amino acid composition of the soya enzyme was compared with that of the animal and bacterial enzymes. Free L-arginine was found at the N-end of soya lactate dehydrogenase.

INTRODUCTION

We have been studying the properties and the structure of plant lactate dehydrogenase NAD^+: lactate oxidoreductase (LDH, EC 1.1.1.27) for a long time. After developing a suitable method for its isolation from plant material[1–3] we found that the LDH has a MW of 140000 and consists of four electrophoretically identical subunits with MWs of 36000. These data have been confirmed by Jervis *et al.*[4]. In the study of the structure of the active centre of soya LDH we have found that the amino acids cysteine[2], tyrosine[5] and, in a catalytic function, histidine[3] take part in the bonding of the coenzyme to the apoenzyme. The present work is another contribution to the study of the structure of plant LDH.

RESULTS

The final preparation of soya LDH was characterized by electrophoresis in a polyacrylamide gel or by affinity electrophoresis. It has been found from the ratio of the electrophoretic mobilities of standard proteins and of soya LDH in media with various pH values (from 3.5 to 10) that the isoelectric point of the enzyme equals 7.0.

The values specifying the amino acid content in soya LDH are related to the relative MW of a subunit, 36000[1]. Each value is the average of three determinations. The contents of valine and methionine have not been corrected for slow liberation during hydrolysis.

The terminal amino acid was labelled by dansylation and determined by 2D-TLC, comparing it with standards. L-Arginine has been found to be the subunit terminal amino acid in soya LDH.

DISCUSSION

To isolate the enzyme from plant tissues, an earlier method[3] was employed with minor modifications. The most effective step in the isolation procedure was affinity chromatography on an AMP–Sepharose column (Table 1). The specific activity of the preparation increased 55 times, a 77% activity being preserved. For the affinity elution of the enzyme from the column, a more concentrated NAD^+ solution (16.5 mmol/l.) was found suitable. In this way a greater recovery was obtained and the enzyme preparation obtained was more concentrated. The homogeneity of the final LDH preparation was studied electrophoretically on polyacrylamide gels, using affinity electrophoresis with polymerically bound Blue Dextran and with isoelectric focussing in Ampholine, pH 3.5–10. None of the techniques employed indicated a heterogeneity in the soya LDH preparation.

The amino acid composition of soya LDH subunit is shown in Table 2. The difference in the contents of various amino acids in the plant, animal[9] and bacterial[10] enzymes are not great. The values of valine and methionine in soya LDH subunit have not been corrected for slow release during the protein hydrolysis and they will probably be higher in soya LDH. The cysteine content in native LDH was determined by the method of ref. [6] using Ellman reagent.

For the determination of the *N*-terminal amino acid, the method of ref. [7] was used without modifications. It has been found that soya LDH is terminated by L-arginine. This result confirms our previous finding[1] that soya LDH consists of four electrophoretically identical subunits; it is also another criterion for homogeneity of the preparation of soya LDH.

In all animal enzymes that have been studied so far, the *N*-terminal was acetylated[8]. Because an acetyl group on the terminal amino acid would prevent dansylation, it can be assumed that the *N*-terminal amino acid is free in the soya enzyme.

EXPERIMENTAL

Materials. The soya seeds (*Glycine max* L.) were obtained from Soja, Kolín, Czechoslovakia; AMP–Sepharose 4B and

Table 1. LDH isolation from germinating soya plants

Fraction	Total activity (units)	Total proteins (mg)	Specific activity (unit/mg)	Purification degree
Crude extract	342.5	1700	0.201	1
30–40% satd Sulphate fraction	109.9	280	0.393	2
Eluate from AMP—Sepharose	84.6	4	21.150	105

Table 2. Amino acid composition of soya LDH subunit

Amino acid	Amino acid residues per 36000 g of protein (mol)
Aspartic acid	29.67 (30)
Threonine	13.80 (14)
Serine	17.56 (18)
Glutamic acid	38.36 (38)
Proline	14.09 (14)
Glycine	33.88 (33)
Alanine	19.68 (20)
Valine	16.48 (16)
Methionine	2.26 (2)
Isoleucine	14.01 (14)
Leucine	22.13 (22)
Tyrosine	1.90 (2)
Phenylalanine	10.42 (10)
Histidine	6.09 (6)
Lysine	16.06 (16)
Arginine	12.49 (12)
Tryptophan	—
Cysteine	5.95 (6)

Numbers in parentheses indicate number of residues expressed to the nearest integer.

Blue Dextran from Pharmacia, Uppsala, Sweden; NAD^+ from Imuna, Šarišské Michalany, Czechoslovakia; and Ampholine from LKB, Sweden.

Enzyme isolation. The extract from 32 hr old germinating soya plants was fractionated with $(NH_4)_2SO_4$. LDH was isolated from the 30–40% satd fraction by affinity chromatography on an AMP–Sepharose 4B column. The enzyme was eluted with a 16.5 mM NAD^+ soln. Detailed data can be found in ref. [3].

Determination of the enzyme activity. The LDH activity was determined from the rate of lactate oxidation, using a method described previously[3]. The activity unit is defined as the enzyme amount that catalyses the formation of 1 μmol pyruvate/min. The protein concn was determined by the method of ref. [11], using BSA as a standard. Electrophoresis in a polyacrylamide gel was carried out according to ref. [12] in Tris–glycine buffer (pH 8.3). Affinity electrophoresis in a polyacrylamide gel with Blue Dextran was described in ref. [13]. Isoelectric focussing was performed on gels with 7.5% polyacrylamide using Ampholine, at pH 3.5–10, by comparison with standards of proteins with known p*I* values. As standards, cytochrome *c* (p*I*, 10.65), ribonuclease (9.45), whale myoglobin (8.3), horse myoglobin (7.3), conalbumin (5.9), β-lactoglobulin (5.34), bovine serum albumin (4.7) and ferritin (4.4) were used.

Amino acid analysis. An amount of 1 mg LDH was hydrolysed for 20 hr in 6 M HCl at 110°. After evaporation of the acid, the amino acid content was determined in the hydrolysate on a Durrum 500 instrument. The *N*-terminal amino acid was determined by the method of ref. [7].

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