Biochimica et Biophysica Acta, 452 (1976) 621—624

BBA Report

BBA 61310

RESOLUTION AND COMPLEMENTATION OF THE LABILE L-LEUCINE-PYRUVATE TRANSAMINASE

AN INTERMEDIATE DURING ENZYME FORMATION UNDER NITROGEN STARVATION IN *GLUCONOBACTER SUBOXYDANS*

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(Received July 30th, 1976)

Summary

L-Leucine-pyruvate transaminase (mol. wt. 70 000) in *Gluconobacter suboxydans* synthesized during nitrogen starvation contained a labile form which changed to the stable one later. The labile enzyme (mol. wt. 70 000) dissociated to the two proteinaceous components: a cationic one (mol. wt. 10 000—20 000) and an anionic one (mol. wt. 50 000—60 000), during column chromatography on DEAE-cellulose. The enzyme activity was reconstructed when they were mixed. The reconstructed enzyme had almost the same molecular size and enzymatic properties as the labile and the native stable enzymes.

We have investigated amino acid metabolism in *Gluconobacter suboxydans*, a unique heterotrophic aerobe which does not have a conventional tricarboxylic acid cycle [1], and found an unusual transaminase, tentatively named L-leucine-pyruvate transaminase [2—5]. The enzyme was found only in bacteria of the genus *Gluconobacter*. It was also demonstrated that the enzyme was rapidly synthesized under nitrogen-free conditions and that the newly synthesized enzyme contained a labile enzyme which is inactivated easily by heat treatment or freezing and thawing [6, 7]. The contents of the stable (i.e. native) enzyme increased during the induction period. These suggested that the enzyme formation proceeded in two steps: the labile enzyme is synthesized at an early stage of induction, and then it converts to the stable form. The failure to separate the labile enzyme from the stable one by gel-filtration on various types of Sephadex indicated that their molecular sizes are almost the same [6]. On the other hand, the labile enzyme activity was lost

after column chromatography on DEAE-cellulose [6]. Thought at first we thought the phenomenon was due to its inactivation during the procedure, it recently became obvious that the labile enzyme resolves into at least two components which do not have enzyme activity during column chromatography, and that the enzyme activity is reconstructed by mixing them.

Cultivation of the microorganism and induction of the enzyme were carried out as described previously [6, 7]. The cell-free extract obtained by the method reported previously [2] was dialyzed against 0.01 M potassium phosphate buffer (pH 7.0) containing 10^{-4} M pyruvate and 10^{-5} M pyridoxal 5′-phosphate and used as an enzyme preparation. Every buffer used here contained these protecting reagents. All procedures were carried out at 5°C. The enzyme activity was estimated by determining α-ketoisocaproate formed by the method of Tayler and Jenkins [8]. The reaction mixture contained 10 mM L-leucine, 10 mM pyruvate, 0.1 mM pyridoxal 5′-phosphate and 100 mM acetate buffer (pH 5.0). The incubation was carried out at 37°C.

The cell-free extract of induced cells was submitted to column chromatography on DEAE-cellulose. As shown in previous papers [3, 4] L-leucine-pyruvate transaminase was not adsorbed by DEAE-cellulose. Experiment A of

TABLE I

RESOLUTION OF THE LABILE L-LEUCINE-PYRUVATE TRANSAMINASE DURING COLUMN CHROMATOGRAPHY ON DEAE-CELLULOSE, RECONSTRUCTION OF THE ENZYME ACTIVITY AND CHARACTERIZATION OF THE COMPONENTS

The transaminase activity is indicated as the amount (μmol) of α-ketoisocaproate formed in the reaction mixture (1 ml) per h.

Enzyme preparation	Enzyme activity	
	(μmol product/h)	(%)
Experiment A:		
Cell-free extracts of glutamate-grown cells[1]	3.40	100
DEAE-cellulose unadsorbed fraction (a)[2]	3.28	94
DEAE-cellulose adsorbed fraction (b)[3]	0.18	4
a + b	3.44	101
Cell-free extracts of induced cells[4]	1.42	100
DEAE-cellulose unadsorbed fraction (c)	0.51	36
DEAE-cellulose adsorbed fraction (d)	0.04	3
c + d	1.31	92
Experiment B:		
Frozen c[5]	0.57	40
Frozen c + d	0.90	64
Frozen c + frozen d	0.88	62
c + frozen d	1.30	92
Frozen d	0.03	2
Boiled c[6]	0.06	4
Boiled c + d	0.02	1
Boiled c + boiled d	0.03	2
c + boiled d	1.16	82
Boiled d	0.01	1

[1] The cell-free extracts were obtained from cells grown on medium containing glutamate as a nitrogen source for 48 h. The enzyme concentration in the reaction mixture corresponded to 0.185 mg/ml protein of the cell-free extract.
[2] The column of DEAE-cellulose equilibrated with 0.01 M potassium phosphate buffer was used. The unadsorbed fraction was dialyzed against the same buffer.
[3] The eluates with the buffer containing 0.25 M NaCl were dialyzed against 0.01 M potassium phosphate buffer.
[4] The cell-free extract was prepared from induced cells. The induction was carried out for 1.5 h at a cell concentration of 0.2 mg/ml. The enzyme concentration in the mixture was 0.133 mg/ml protein as a cell-free extract.
[5] In 12 h, each fraction was twice frozen at −20° C and thawed at room temperature.
[6] Each fraction was immersed in boiling water for 3 min.

Table I indicated that the stable enzyme activity was recovered in the unadsorbed fraction, while the labile enzyme activity was lost during chromatography, and no activity was found in eluates at higher ionic concentration. However, full restoration of the transaminase activity was observed upon addition of the DEAE-cellulose 0.25 M NaCl fraction to the unadsorbed fraction. This is characteristic in the cell-free extracts of the induced cells. The properties of the fractions were investigated (Experiment B of Table I). The DEAE-cellulose unadsorbed fraction was unstable. The enzyme activity in the unadsorbed fraction was dependent on the stable enzyme and remained after freezing and thawing while the ability to form the enzyme activity was lost after the same treatment. They were completely inactivated by heating at 100°C for 3 min. The DEAE-cellulose adsorbed fraction was more stable against freezing and thawing and also showed the ability to form enzyme activity even after heat treatment for 3 min. However, 15-min heat treatment caused complete loss of the activity. It was also inactivated by digestion with some proteolytic enzymes. Judging from the results mentioned above, it is obvious that the two fractions have proteinaceous properties.

Fig. 1 shows the results of gel filtration of the cell-free extracts of the induced-cell and DEAE-cellulose fractions on Sephadex G-75. The stable enzyme activity was measured after freezing and thawing. The elution positions of the total and the stable enzyme activities were almost the same. This suggested that the stable and the labile enzyme have the same molecular size (V_e/V_0 = 1.18, Fig. 1A). The enzyme assay during column chromatography of the two DEAE fractions was carried out with and without the addition of

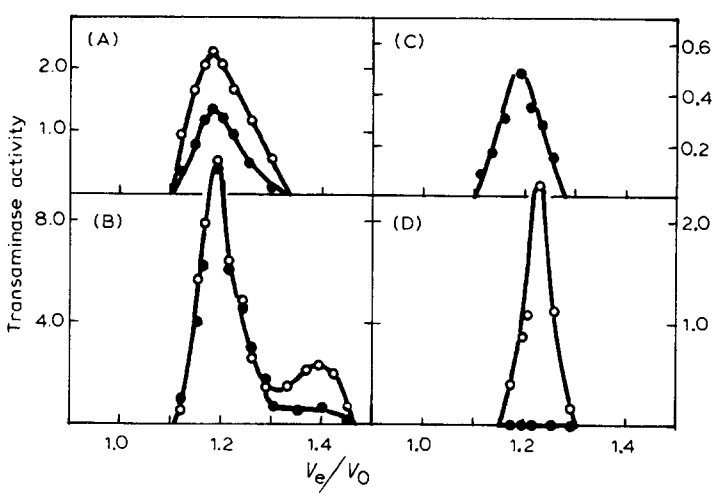

Fig. 1. Gel filtration of various enzyme preparations on Sephadex G-75. A column (2.5 x 120 cm) of Sephadex G-75 was used. The enzyme preparations used were: (A) Cell-free extracts of the induced cells. The stable enzyme activity (●—●) was estimated after the fractions had been frozen twice at −20° C and thawed at room temperature. The reactions were carried out under the standard conditions. The total activity is indicated by ○—○. (B) DEAE-cellulose unadsorbed fraction. The enzyme activities were measured under standard conditions (●—●) or with the addition of eluate from (D) (○—○). (C) Mixture of DEAE-cellulose adsorbed and unadsorbed components. The mixture of the eluates from (B) and (D) was submitted to filtration. The enzyme activities were measured under the standard conditions. (D) DEAE-cellulose adsorbed fraction. Enzyme activities were measured under the standard conditions (●—●) or with the addition of eluate from (B) (○—○). The transaminase activity is indicated as α-ketoisocaproate formed (μmol/h per ml of eluate).

the other component (see the legends of the figure). In the case of the DEAE-cellulose unadsorbed fraction, a component which forms the enzyme activity with DEAE-cellulose adsorbed fraction eluted from the column later than the stable enzyme in the fraction (V_e/V_0 = 1.39 and 1.19, respectively, Fig. 1B). The molecular size of the component in the DEAE-cellulose adsorbed fraction seems to be smaller than that of the stable enzyme (V_e/V_0 = 1.22, Fig. 1D). The gel filtration profile of the mixture of the components obtained in Fig. 1B and 1D indicated that the elution position of the reconstructed enzyme activity was the same as that of the stable enzyme (V_e/V_0 = 1.19, Fig. 1C). Enzymatic properties such as optimum pH and substrate specificity were almost the same as those of the stable transaminase. The molecular weight of L-leucine-pyruvate transaminase has already been reported as 70 000 [4]. From their elution positions, the molecular sizes of the components of DEAE-cellulose adsorbed and unadsorbed fractions were 50 000—60 000 and 10 000—20 000, respectively.

From the data in this report, it was suggested that the labile enzyme (mol. wt. 70 000) formed under nitrogen starvation dissociates easily to the labile and cationic component (mol. wt. 10 000—20 000) and the stable and anionic one (mol. wt. 50 000—60 000). The two components have proteinaceous properties and the ability to reconstruct the complex which shows enzyme activity. The reconstructed complex has the same molecular size as the native enzyme. Studies on the subunit structure of L-leucine-pyruvate transaminase have not been made yet. It is necessary to investigate further the structural differences between the two types of the enzyme and the mechanisms of stabilization of the labile enzyme.

We wish to thank Dr. M. Moriguchi, Associate Professor of Oita University and Dr. R. Sasaki, Associate Professor of Kyoto University, for their useful suggestions.

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