DISSOCIATION PROPERTIES OF ASPARTOKINASE I-HOMOSERINE DEHYDROGENASE I EXTRACTED FROM A MISSENSE MUTANT OF E.coli K12

Jacques THEZE and Michel VERON
Service de Biochimie Cellulaire,
Institut Pasteur, 75015 Paris, France.

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SUMMARY. The threonine sensitive aspartokinase-homoserine dehydrogenase devoid of aspartokinase activity has been extracted from a missense mutant of E. coli K12 and some of its properties have been investigated. The genetic localization of the corresponding mutation indicated that the amino acid replacement lies in the kinase region of the molecule. The cooperativity of threonine inhibition of the homoserine dehydrogenase activity is lowered. The measurement of the molecular weight of the enzyme in presence or absence of threonine indicates that the molecule dissociates more easily than the wild type enzyme. These results are discussed in view of the recent structural model proposed for aspartokinase I-homoserine dehydrogenase I.

Aspartokinase I-homoserine dehydrogenase I (E.C. 2.7.2.4. and E.C. 1.1.1.3), a bifunctional enzyme involved in threonine biosynthesis, is composed of four identical subunits (M.W. = 86000) (1). Each subunit possesses the catalytic sites for the two activities which have been shown to be carried by two independent regions of the polypeptide chain (2). Moreover it has been shown that thrA, the structural gene coding for aspartokinase I-homoserine dehydrogenase I is composed of two cistrons $thrA_1$ and $thrA_2$ coding for the aspartokinase I and the homoserine dehydrogenase I activities respectively (3). A model has been recently proposed for the structure of aspartokinase I-homoserine dehydrogenase I (4) in which the aspartokinase globules play a central role in the organization of the tetrameric structure of the enzyme.

In this communication, we describe the threonine sensitive homoserine dehydrogenase devoid of aspartokinase activity extrac-

ted from strain Gif 106M1. The genetic characterization of the mutant and some of its dissociation properties are presented.

MATERIALS AND METHODS

Bacterial strains.

Gif 106M1 has been previously selected on the basis of homoserine and diaminopimelic acid auxotrophy in a strain devoid of aspartokinase II homoserine dehydrogenase II and aspartokinase III (5). Strain HfrH is from the collection of the Service de Biochimie Cellulaire.

Buffers and measurement of activity.

Crude extracts were prepared in buffer A : 20 mM potassium phosphate pH 7.2, 2 mM magnesium titriplex, 150 mM potassium chloride and 1 mM dithiothreitol. When indicated, this buffer also contained 2 mM L-threonine(buffer AT).

Homoserine dehydrogenase activity was measured in the physiological direction using NADPH and aspartate semialdehyde as substrates (6). Aspartokinase activity was estimated using the hydroxamate test (7).

Growth of strain HfrH and Gif 106M1 and preparation of the crude extracts.

Overnight cultures, grown on minimal medium were diluted in the same medium to 10^8 bacteria/ml. After three generations, the bacteria were harvested, washed and disrupted by sonic oscillation in buffer A or AT depending on the experiment (see below). For strain Gif 106 M1, the medium was supplemented with D,L-isoleucine 2×10^{-3} M, D,L-arginine 2×10^{-3} M, D,L-homoserine 3×10^{-4} M, L-lysine 10^{-3}M and meso-diaminopimelic acid 10^{-4}M.

Molecular weight determination.

The molecular weight of the aspartokinase -homoserine dehydrogenase was determined by gel filtration on a Sephadex G-200 column (ϕ 1.5 cm ; length 70 cm) equilibrated at room temperature with the appropriate buffer. The column had been previously calibrated with the following proteins : beef liver catalase (M.W. = 240,000), yeast alcohol dehydrogenase (M.W. = 150,000), pig heart malate dehydrogenase (M.W. = 70,000) and cytochrome c (M.W. = 12,000). In each experiment alcohol dehydrogenase was added as an internal marker.

RESULTS

Genetic localization

The mutation thrA 1101 carried by Gif 106M1 has been previously localized in the threonine cluster of E.coli K12 by transduction (8). As it reverts easily, it must be considered as a point mutation. In addition the complementation analysis performed as previously described (3) showed that thrA 1101 is a missense mutation localized in the $thrA_1$ cistron.

Threonine inhibition.

No detectable difference was found between the specific activity of the homoserine dehydrogenase measured in crude extracts of Gif 106M1 and HfrH. This is expected because of the independence of the aspartokinase and homoserine dehydrogenase globules with respect to their catalytic functions. The Hill number of the cooperativity of threonine inhibition of this remaining activity is $\bar{n} = 1.6$, significantly lower than the value of $\bar{n} = 3$ found with the wild-type (9).

Analysis of the molecular weight.

In a first set of experiments, the apparent molecular

weights of the protein from HfrH and Gif 106M1 were compared in the presence of 2 mM L-threonine. The elution profiles are shown in Fig. 1 (part A and B). Seventy percent of the activity from

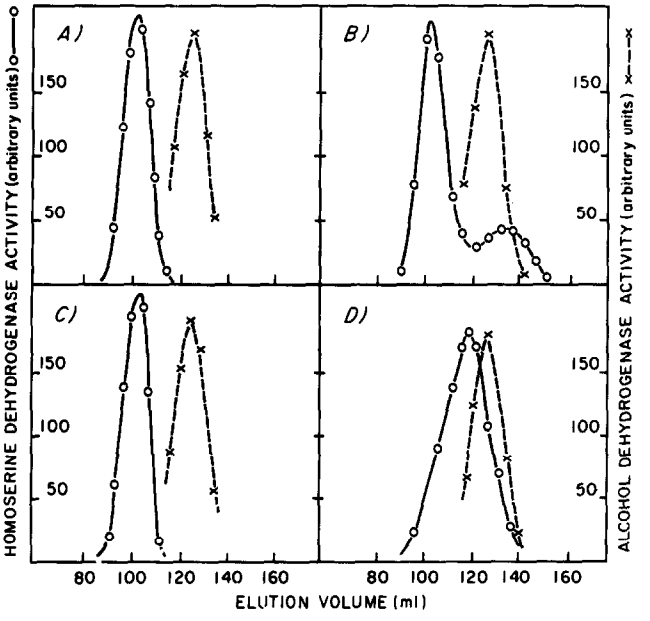

Fig. 1. Gel filtration on Sephadex G-200. The following crude extracts were analyzed :

A) HfrH in presence of threonine 2 mM
B) Gif 106M1 in presence of threonine 2 mM
C) HfrH in absence of threonine
D) Gif 106M1 in absence of threonine.

Gif 106M1 was eluted in the same volume as the activity of HfrH. This corresponds to a molecular weight of 350,000. The homoserine dehydrogenase from Gif 106M1 is thus mostly in a tetrameric form in the presence of threonine as is the enzyme from the wild-type. However, the crude extract of Gif 106M1 contains also a small amount of a lighter active homoserine dehydrogenase (M.W. = 85,000). This protein which was eluted in the region corresponding to the

aspartokinase I-homoserine dehydrogenase I monomer, is no longer sensitive to threonine inhibition.

In the absence of threonine, the elution pattern was very different (Fig. 1, part C and D). Whereas the enzyme from the wild type was still eluted as a tetramer, the homoserine dehydrogenase activity extracted from Gif 106M1 was eluted as a single peak corresponding to a molecular weight of roughly 170,000. The activity remains sensitive to threonine inhibition.

DISCUSSION

The homoserine dehydrogenase extracted from the mutant strain Gif 106M1 presents dissociation properties which are different from those of the enzyme extracted from the wild type strain HfrH. Whereas the latter is a tetramer in crude extracts, the protein extracted from the mutant dissociates either in the absence or in presence of threonine, a ligand usually known to stabilize the tetrameric structure (10).

It is worth noting that a species MW 85,000 is observed. The presence of this molecule seems to indicate that the monomer can retain the homoserine dehydrogenase activity. However, no definitive argument can be drawn from this experiment since a reassociation of the subunits under the test conditions cannot be excluded.

The genetic data indicate that the amino acid replacement lies in the kinase region of the protein. Not only it does inactivate the kinase activity, but also it may alter the association areas between the subunits. The low cooperativity of threonine inhibition is consistent with this conclusion. These data point out

the correlation between alteration of the aspartokinase globules and dissociation ability, a result which is in agreement with the structural model previously proposed (4).

An enhanced dissociability of the aspartokinase I-homoserine dehydrogenase I has also been found in mutant strains where the enzyme is desensitized towards threonine (9). In view of our results, it should be of interest to correlate the dissociation properties of these mutants with their genetic localization in the thrA gene.

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