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E.coli aspartokinase II-homoserine dehydrogenase II polypeptide chain has a triglobular structure

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E.coli aspartokinase II-homoserine dehydrogenase II is, as aspartokinase I-homoserine dehydrogenase I, composed of three globular domains: the N-terminal domain is endowed with kinase activity; the C-terminal domain carries the dehydrogenase activity. These two parts of the polypeptide chain are separated by a central inactive domain. Thus, the polypeptide chains of the two multifunctional proteins are homologous not only in their sequence but also in their triglobular domain structure.

Using limited proteolysis, we have shown (1,2) that the monomer of the homotetrameric aspartokinase I-homoserine dehydrogenase I (AK I-HDH I) (Mr = 4 x 89,000) consists of three non-overlapping compact regions and their role in the properties of native AK I-HDH I has been found: a 27 kilodalton fragment exhibiting residual aspartokinase activity, as a monomeric species, on the N-terminal side of the native polypeptide; a central 25 kilodalton fragment, able to associate itself as a stable dimeric structure, implicated in some of the contacts responsible for the polymeric nature of AK I-HDH I; and finally, a monomeric active homoserine dehydrogenase fragment of Mr = 33 kilodalton (2).

A previous limited proteolysis study of the homodimeric aspartokinase II- homoserine dehydrogenase II (AK II-HDH II; Mr = 2 x 88,000) has shown that its constitutive polypeptide consists at least of two non-overlapping domains: whereas the smaller proteolytic fragment (2 x 24,000) is devoid of both enzymatic activities, the larger fragment (2 x 37,000) is endowed with full homoserine dehydrogenase activity (3). In this study, an active aspartokinase fragment was not recovered and

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the exact location of the dehydrogenase and of the inactive fragment was not determined.

On the other hand, we have recently compared the primary sequences (4,5) of the two E.coli bifunctional proteins and have concluded that AK I-HDH I and AK II-HDH II derive from a common ancestor (5). The detailed comparison of the sequences, added to the immunological cross-reactivity pattern of the denatured fragments (6) leaves no doubt that in AK II-HDH II also, the kinase activity resides in the N-terminal moiety and the dehydrogenase activity in the C-terminal moiety of the polypeptide chain. The results presented here define a fragment of AK II-HDH II endowed with aspartokinase activity.

Materials and Methods

Aspartokinase II-homoserine dehydrogenase II has been obtained in the pure state from cells of the E.coli K12 mutant (Gif 881), constitutive for the enzymes of the methionine regulon. The method used for purification differs slightly from that previously described (7) in that the DEAE-Sephadex A50 step was introduced right after the ammonium sulfate precipitation and in that the ammonium sulfate extraction was followed in order by an Ultrogel AcA34 filtration and by a new DEAE-Sephadex A50 chromatography.

Aspartokinase was measured by the coupled assay described by Wampler and Westhead (8) and homoserine dehydrogenase was tested in the forward direction at pH 7.2 (9). Proteolysis was carried out in a potassium phosphate buffer pH 7.2 containing 2 mM MgK_2 EDTA and 10 mM dithiothreitol, at 27°C, by pronase type VI of Streptomyces griseus (2) (0.5 p.100 w/w) acting on AK II-HDH II (0.3-1 mg/ml). It was interrupted by addition of 4 mM phenylmethane sulfonate (in 2-propanol). When the proteolysis was performed in the absence of ligand, KCl (150 mM), L-aspartate (10 mM) and DL-homoserine (10 mM) were added to the aliquots.

Protein samples were analyzed on 7.5 p.100 polyacrylamide gels according to Davis (10), colored with Coomassie Blue type G (0.04 p.100 in 3.5 p.100 perchloric acid) and destained by 5 p.100 acetic acid. SDS polyacrylamide gels were run according to Laemmli (11).

Results and Discussion

In the previous study (3), it was observed that aspartate plus homoserine slow down considerably the proteolysis: the fragment characterized were the products of the proteolysis carried in the presence of these two ligands. Furthermore, the proteolysis mixture was analyzed when aspartokinase activity had reached between 1 and 5 p.100 of its

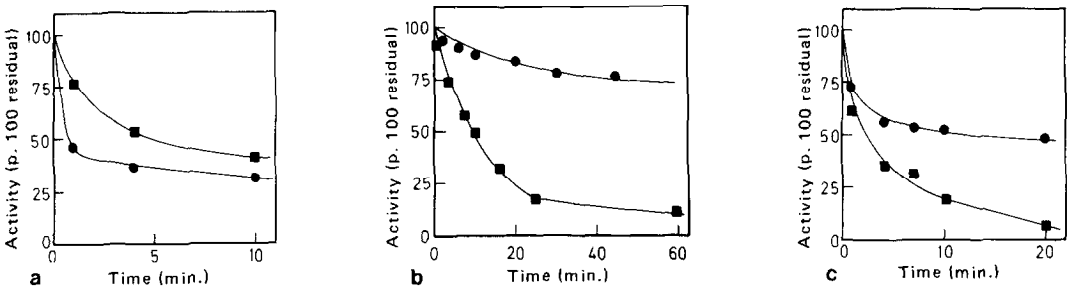


Figure 1a : Kinetics of proteolysis of AK II-HDH II in the presence of L-aspartate.
AK II-HDH II: 0.43 mg/ml; L-aspartate 10 mM; 0.5% pronase/AK II-HDH II; (●) dehydrogenase activity; (■) kinase activity.

Figure 1b : Kinetics of proteolysis of AK II-HDH II in the presence of L-homoserine.
Same proteolysis conditions as in Fig. 1a, but L-aspartate is replaced by L-homoserine 10 mM; same symbols as in Fig. 1a.

Figure 1c : Kinetics of proteolysis of AK II-HDH II in the absence of ligands.
AK II-HDH II: 0.32 mg/ml; same symbols as in Fig. 1a.

original value. We have found now that aspartate protects preferentially the kinase, whereas homoserine is an excellent protector of the dehydrogenase (Fig. 1a and 1b). We have deliberately chosen to analyse the nature of the fragments formed at different times of proteolysis carried without the addition of any ligand (Fig. 1c). The analysis was performed by SDS-polyacrylamide gel electrophoresis (Fig. 2). There are obviously three proteolytic fragments respectively called 1, 2 and 3. Fragment 1 (ca 40,000) is similar to that already obtained in the previous study (3), persists throughout the proteolysis and corresponds to the dehydrogenase domain of AK II-HDH II. Fragment 3 (Mr = 26,000) also persists until the end of the proteolysis and may correspond to the inactive dimeric fragment (3) (Mr = 24,000).

Fig. 2 shows that during proteolysis in the absence of ligands, the entire polypeptide chain disappears faster than the aspartokinase activity (see Fig. 1c). This result suggests that this activity is carried by an aspartokinase fragment (Mr = 27,000) corresponding to fragment 2 whose disappearance coincides with the loss of aspartokinase activity. Elution

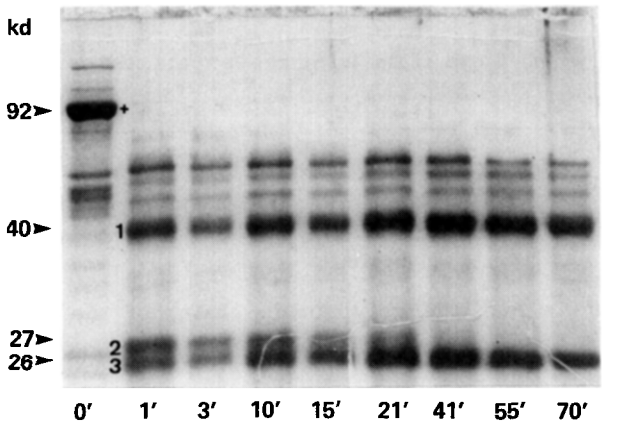


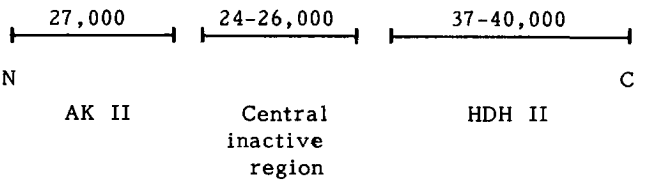
Figure 2 : Polyacrylamide gel electrophoresis in the presence of SDS of the proteolysis product of AK II-HDH II.

AK II-HDH II (1 mg/ml) was proteolyzed with pronase (1 p.100 W/W). Proteolysis conditions are identical to those of Fig. 1c 60 µl samples were analyzed at different times of protolysis. The figures at the left are the molecular weight of known markers. +: monomer of AK II-HDH II; 1, 2 and 3 are the proteolytic fragments described in the text.

from native gels (with the buffer described under Materials and Methods containing in addition 150 mM KCl) shows that only one band exhibits aspartokinase activity. This band corresponds to a protein of a molecular weight lower than 50,000 by the method of Hedrick and Smith (12) and it can not be yet concluded whether the aspartokinase active fragment is monomeric or dimeric.

The fragment endowed with homoserine dehydrogenase activity has the same C-terminus as the native enzyme (Leu-Leu-COOH; ref. 13). The aspartokinase domain of AK I-HDH I is N-terminal (1,2) and we have recently obtained the N-terminal sequence of the 10 first residues of the 27 kilodaltons AK I fragment of AK I-HDH I, which is identical to that of the native protein (Véron, Guillou and Cohen, in preparation). Comparison of the sequences of AK I-HDH I and AK II-HDH II (5) has shown that the two proteins share a common ancestor and in particular that the N-terminal domains of the two proteins are highly homologous. Since inspection of Fig. 2 rules out any precursor-product relationship between fragments 2

and 3, we are led to propose the following organisation for AK II-HDH II, which is the only one compatible with the experimental data:



The conclusion is that the polypeptide chains of the two multifunctional proteins are homologous not only in their sequence (5) but also in their triglobular domain structure.

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