INTERACTION BETWEEN PHENYLALANINE-tRNA AND THE ALLOSTERIC FIRST ENZYME OF THE AROMATIC AMINO ACID BIOSYNTHETIC PATHWAY

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There is some evidence that certain amino acids must be activated in order to repress the formation of their own biosynthetic enzymes /Schlesinger and Magasanik, 1964; Eidlic and Neidhardt, 1965; Roth and Ames, 1966; Freundlich, 1967/. Another group of indirect evidence suggests that the allosteric enzymes of several biosynthetic pathways might play some role in the repression process /Sommerville and Yanofsky, 1965; Berberich and Gots, 1965; Kovách et al., 1968/. Supposing that both the aminoacyl-tRNA and the allosteric enzyme are required for the repression of the enzymes of an amino acid biosynthetic pathway, it seems to be possible that the allosteric enzyme - aminoacyl-tRNA complex is the repressor itself. The first step to test this hypothesis would be the detection of the formation of such a complex. In this paper we report that one of the allosteric first enzymes of the aromatic amino acid biosynthetic pathway in E. coli, the phenylalanine-sensitive 3-deoxy-D-arabino-heptulosonate-7-phosphate /DAHP/-synthase /EC 4.1.2.15/ combines specifically with phenylalanine-tRNA. The formation of this complex was demonstrated by running the mixture of the two components on a Sephadex G-75 column.

Methods. The phenylalanine-sensitive DAHP-synthase /isoenzyme 1a/ was purified from Escherichia coli K 12. Its molecular weight is approximately 1.6×10^5 /Staub and Dénes, 1968/ and its K_i for phenylalanine 3.5×10^{-5} M /Staub and Dénes, 1967/. In these experiments a 200 fold purified enzyme preparation was used /specific activity: 0.81 µmole DAHP formation per min. per mg protein/.

tRNA was isolated from E. coli B /Zubay, 1966/. The tRNA-s were "stripped" according to Carbon and Curry /1968/.

The "stripped", unfractionated tRNA was charged with ^{3}H-phenylalanine, or ^{3}H-, or ^{14}C-valine /see Legends/ with a crude amino acid activating enzyme preparation from E. coli B /Münch and Berg, 1966/. After charging, free amino acid and protein were removed by phenol and Sephadex G-25 treatment.

Radioactivity was measured in a Packard scintillation spectrometer. In double labeling experiments the ^{14}C and ^{3}H radioactivities were calculated by using the external channels ratio method.

Results. As shown in Fig.1. the enzyme and the tRNA are completely separated on Sephadex G-75. The small front peak on Part B is due to the presence of contaminating rRNA in the tRNA preparation.

Fig.2. shows the result of an experiment in which tRNA charged with ^{3}H-phenylalanine was mixed with the enzyme and run on Sephadex G-75. It can be seen that a large amount of radioactivity /about 10 per cent of the input, or 40 per cent of the intact phenylalanine-tRNA/ runs together with the enzyme. The third peak of radioactivity is probably due to the free ^{3}H-phenylalanine formed during the experiment. One could argue that the enzyme-bound radioactivity is not phenylalanine-tRNA but free

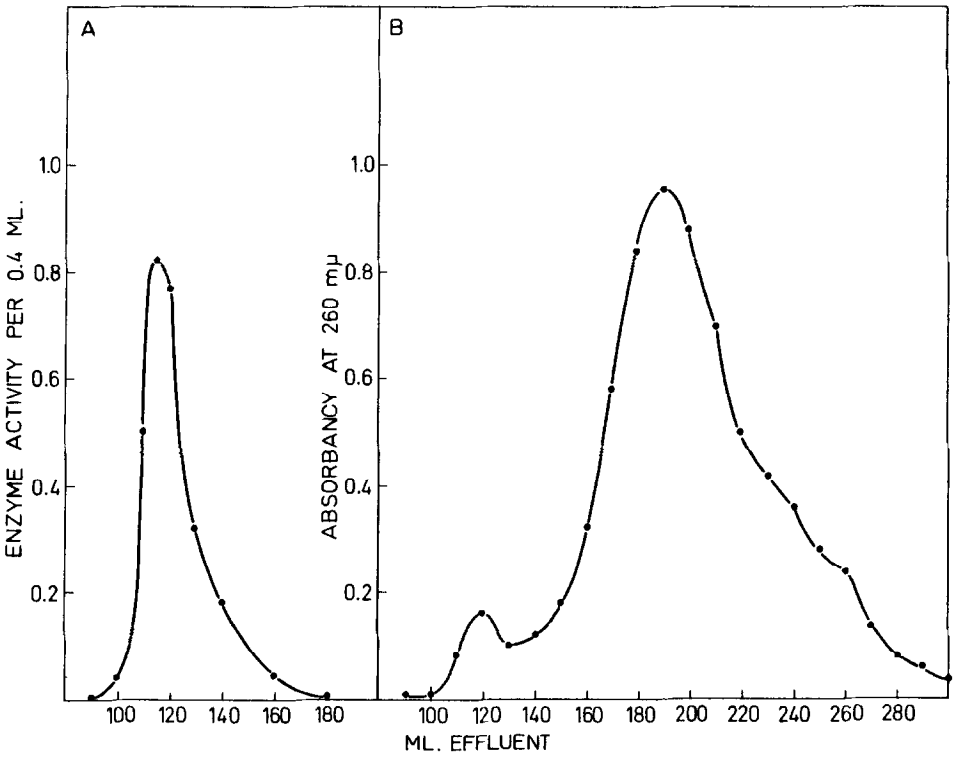

Fig.1. Chromatography of purified DAHP-synthase and tRNA on Sephadex G-75. Column size: 37 x 350 mm. Elution buffer: 0.05 M NaCl in 0.05 M phosphate /pH 6.5/. Temperature: 2 - 4°. Flow rate: 60 ml/hour. Part A: 2 mg DAHP-synthase. Part B: 4 mg tRNA.

TABLE I.

The valine- and phenylalanine-acceptor ability of enzyme-bound and unbound tRNA. 1.5 μC ^{3}H-phenylalanine and 0.25 μC ^{14}C-valine /specific activity: 92 mC/mM/ were used in each assay in a final volume of 1 ml. The enzyme-bound tRNA used in an assay represented approximately one tenth of the total tRNA of that fraction. The free tRNA was 1 mg per assay mixture /approximately 5 per cent of the total/.

	^{3}H dpm	^{14}C dpm	$^{3}H/^{14}C$
enzyme-bound tRNA	4730	1030	4.59
free tRNA	28330	100610	0.28

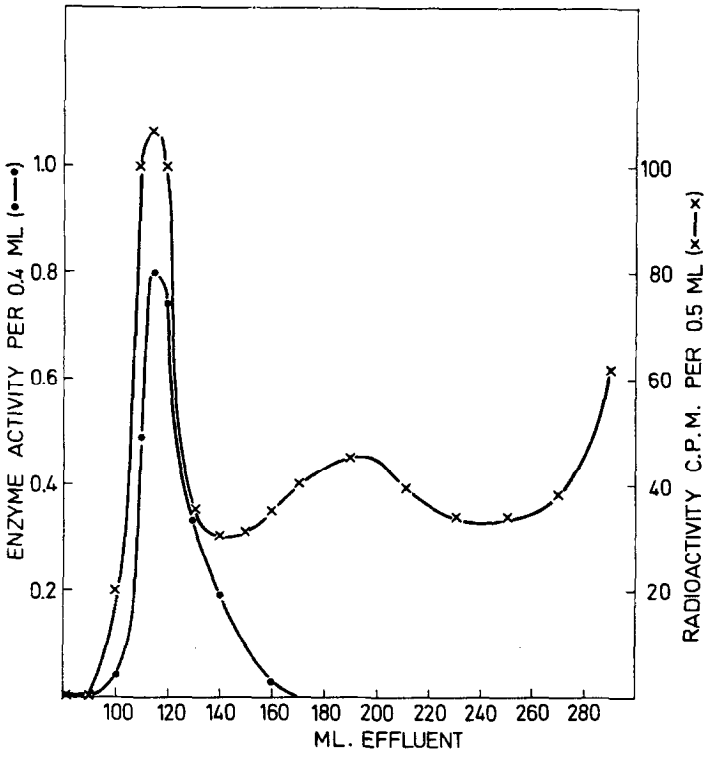

Fig.2. Chromatography of DAHP-synthase with tRNA charged with ^{3}H-phenylalnine on Sephadex G-75. 10 mg tRNA was charged with 100 µC ^{3}H-phenylalanine /specific activity: 1013 mC/mM/. After phenol and Sephadex G-25 treatment the tRNA contained about 4 x 10^{4} cpm radioactivity. It was mixed with 2 mg DAHP-synthase in the presence of 10^{-2} M unlabeled Phe. Volume 2 ml.

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phenylalanine bound to the allosteric site of DAHP-synthase. However, the excess unlabeled phenylalanine, added to the mixture /see Legend/ rules out this possibility. It can be calculated that in the presence of 10^{-2} M unlabeled phenylalanine, the radioactivity of phenylalanine bound to the enzyme on the feed-back sensitive site could not be higher than 0.5 per cent of the observed amount of bound radioactivity.

In order to test the specificity of the binding, a similar experiment was performed with valine instead of phenylalanine.

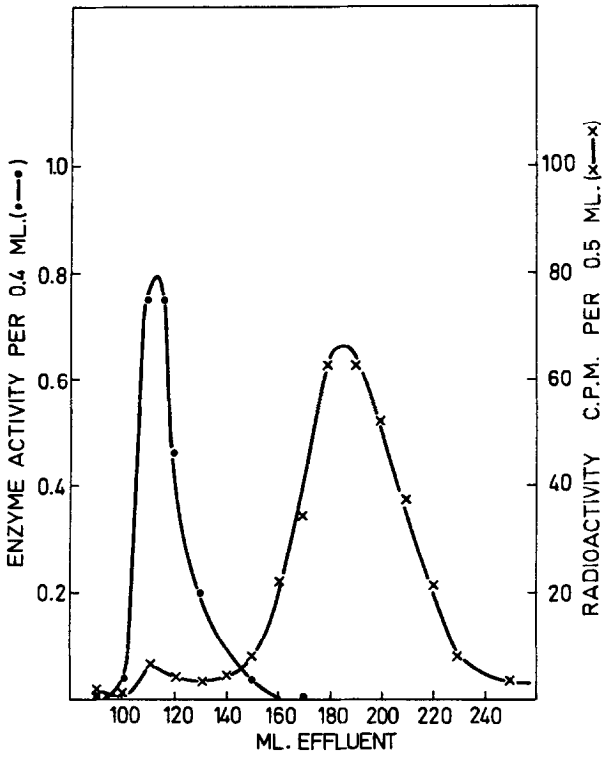

Fig.3.

Chromatography of DAHP-synthase with tRNA charged with ^{3}H-valine. 10 mg tRNA was charged with 100 µC ^{3}H-valine /specific activity: 276 mC/mM/. After purification the tRNA contained about 1.6×10^{4} cpm radioactivity. It was mixed with 2 mg DAHP-synthase in a volume of 2 ml.

Fig. 3. illustrates that in contrast to the previous experiment, valyl-tRNA did not bind to the enzyme /less than 1 per cent of the input, less than 3 per cent of the intact valyl-tRNA/.

Experiments with ^{32}P-labeled tRNA indicated that the amount of tRNA, bound to DAHP-synthase is higher than to be expected on the basis of the ^{3}H-phenylalanine charged tRNA experiments. We assumed therefore, that not only the charged but also the uncharged form of $tRNA^{Phe}$ can be bound to the enzyme. The specificity of this binding was confirmed by the following

experiment. 20 mg "stripped" tRNA was chromatographed with 4 mg of enzyme on Sephadex G-75 as described. The fractions containing the enzyme /plus bound tRNA/ and those containing the unbound tRNA were separately pooled, freeze-dried and treated with phenol and Sephadex G-25. These two tRNA fractions were then assayed for valine and phenylalnine acceptor activity. Table I. summarizes these data.

The results show that the bound tRNA fraction contains only very small amount of valine acceptor tRNA /approximately 0.5 per cent of the total/ but a considerable part of the total phenylalanine acceptor tRNA /approximately 8-9 per cent/.

Summary. The experiments described in this report demonstrate that the first /allosteric/ enzyme of the common pathway of the aromatic amino acid biosynthesis, the phenylalanine-sensitive DAHP-synthase strongly and specifically binds phenylalanyl-tRNA, either charged with amino acid, or uncharged. A complex of charged tRNA with the allosteric enzyme would be a likely candidate for the role of the repressor in this system. Work is in progress to investigate this possibility.

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