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INTERACTION OF YEAST ARGINYL-tRNA SYNTHETASE AND ASPARTYL-tRNA SYNTHETASE WITH BLUE-DEXTRAN SEPHAROSE : ASSIGNEMENT OF THE BLUE-DEXTRAN BINDING SITE ON THE SYNTHETASES.

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SUMMARY

Yeast arginyl-tRNA synthetase and aspartyl-tRNA synthetase like nucleotidyl transferases previously investigated interact with the Blue-Dextran-Sepharose affinity ligand through their tRNA binding domain : the enzymes are readily displaced from the affinity column by their cognate tRNAs but not by ATP or a mixture of ATP and the cognate amino acid in contrast to other aminoacyl-tRNA synthetases. In the absence of Mg^{++}, the arginyl-tRNA synthetase can be dissociated from the column by $tRNA^{Asp}$ and $tRNA^{Phe}$ which have been shown to be able to form a complex with the synthetase, but in presence of Mg^{++} the elution is only obtained by the specific tRNA.

The procedure described here can thus be used : (i) to detect polynucleotide binding sites in a protein ; (ii) to estimate the relative affinities of different tRNAs for a purified synthetase ; (iii) to purify an aminoacyl-tRNA synthetase by selective elution with the cognate tRNA.

INTRODUCTION

We have previously developped a method of affinity chromatography for the study of enzymes which display a high affinity for the nucleic acids (1). One of the ligands used in such affinity chromatography is a semi-specific compound, the Blue-Dextran agarose, which can mimic a polynucleotide. Investigation of E.coli tryptophanyl-tRNA synthetase (1,2) and nucleotidyl-transferase by Deutscher and Masiakowski (3) have shown that such enzymes bound on Blue-Dextran-Sepharose column are dissociated by tRNA. Moe and Piszkiewicz (4) have reported that isoleucyl-tRNA synthetase could be dissociated from Blue-Dextran-Sepharose with ATP or a mixture of ATP and isoleucine as eluant but only in the presence of Mg^{++}. Furthermore they have demonstrated (5) that Blue-Dextran alone behaves as a competitive inhibitor with respect to $tRNA^{Ile}$ for this synthetase.

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In this paper, we report the interaction between arginyl-tRNA synthetase and aspartyl-tRNA synthetase from baker's yeast and Blue-Dextran-Sepharose.

The results of the present work show that the binary complex enzyme-Blue-Dextran-Sepharose is only dissociated by the tRNAs.

This property is utilized for the purification of yeast arginyl-tRNA synthetase.

MATERIAL AND METHODS

Chemicals

Chemicals were obtained from the following sources : Blue-Dextran and cyanogen-bromide-activated Sepharose 4B from Pharmacia (Uppsala, Sweden) ; labelled L-amino acids from the C.E.A. (Saclay, France) ; bovine serum albumin from Sigma (St. Louis, USA) ; dithiothreitol from Calbiochem (Los Angeles, USA) ; ATP (sodium salt) from P.L. Biochemicals Inc. (Milwaukee, USA) ; unfractionated yeast tRNA from Boehringer (Mannheim, Germany).

Purification of tRNAs and aminoacyl-tRNA synthetases

The preparations of pure yeast tRNAs were previously described : $tRNA^{Phe}$ (6), $tRNA_3^{Arg}$ (7), $tRNA_2^{Val}$ (8), $tRNA^{Asp}$ (9). $tRNA^{Ser}$ was a kind gift from Dr. G. Pixa and was prepared by counter-current distribution (6) followed by two BD-cellulose columns. The first was eluted by a NaCl gradient (9) ; in the second chromatography this gradient was followed by elution with 2 M NaCl, 20% ethanol. Arginyl-tRNA synthetase and aspartyl-tRNA synthetase from baker's yeast were prepared as previously described (10,11). Partially purified fractions obtained after DEAE-cellulose column step were used. These fractions had a specific activity of about 500 and 14 units/mg of protein for arginyl-tRNA synthetase and aspartyl-tRNA synthetase respectively. One unit of enzyme catalyses the amino-acylation of 1 nmole of tRNA in 1 min at 37°C under the conditions described below.

Assay

The arginyl-tRNA synthetase activity was measured by the rate of aminoacylation of tRNA as previously described (10).
The other aminoacyl-tRNA synthetase activities were measured under similar conditions according to Kern and coll. (12).

Protein concentrations

Protein concentrations were determined by the micro technique of Schaffner and Weissman (13) using the bovine serum albumin as a standard.

Affinity chromatography

Blue-Dextran-Sepharose column preparation and conditions of affinity chromatography were previously described (1) ; about 10-12 mg of Blue-Dextran were covalently bound per g of dry Sepharose 4B. All the chromatographic steps were carried out at room temperature in sterile disposable syringes.

RESULTS

Binding of arginyl-tRNA synthetase to Blue-Dextran-Sepharose

Arginyl-tRNA synthetase was retained on the Blue-Dextran-Sepharose when it was loaded in a low ionic strength buffer (50 mM Tris-HCl pH 7.5). The enzyme was

eluted from the column at about 200 mM KCl. Neither L-arginine at 0.1 mM nor ATP at concentrations up to 10 mM could dissociate the ArgRS-Blue-Dextran complex.

Dissociation of the binary complex by tRNA

On the contrary, the cognate $tRNA_3^{Arg}$ dissociates the arginyl-tRNA synthetase Blue-Dextran-Sepharose complex. At a concentration of A_{260} = 0.1 (0.16 µM) $tRNA_3^{Arg}$ displaces the arginyl-tRNA synthetase from the column (Fig. 1). Furthermore, the dissociation of the binary complex could be obtained with unfractionated yeast tRNA (Fig. 2). But at the same concentration (A_{260} = 0.1) only 30 per cent of bound arginyl-tRNA synthetase was recovered from the column. More than a ten fold concentration was necessary to obtain a complete elution of the enzyme. However, the difference of ten times in concentrations of cognate $tRNA^{Arg}$ and total tRNA is not high enough to explain the elution of the amino-acyl-tRNA synthetase by its cognate $tRNA^{Arg}$ contained in the unfractionated tRNA, because the content of the three isoacceptor $tRNAs^{Arg}$ does not exceed 5.1% of the total tRNA (7). This suggests that the relatively efficient elution of arginyl-tRNA synthetase by unfractionated tRNA may be due to the presence of both $tRNA^{Arg}$ and of non-cognate tRNAs having a comparable affinity for the enzyme. In fact, it has been shown that $tRNA^{Asp}$ and $tRNA^{Phe}$ could interact with arginyl-tRNA synthetase (14, and J. Gangloff's unpublished results). Assays were then performed with several purified tRNAs including these two species.

As shown in Table 1, in absence of $MgCl_2$, at the low concentration of 0.16 µM where the cognate $tRNA_3^{Arg}$ efficiently dissociates the complex arginyl-tRNA synthetase - Blue-Dextran-Sepharose, $tRNA^{Asp}$ and $tRNA^{Phe}$ eluted also partially the arginyl-tRNA synthetase activity from the column. Nevertheless, when the concentration of tRNA increased, not only those tRNAs which can form a complex with the arginyl-tRNA synthetase could displace the enzyme, but even $tRNA^{Val}$ and $tRNA^{Ser}$ could dissociate 30% of the complex. In the presence of 5 mM of Mg^{2+} however, all these non-cognate tRNAs could no more displace the arginyl-tRNA synthetase from the Blue-Dextran, only the $tRNA_3^{Arg}$ could dissociate the complex albeit the elution was not complete at this concentration of tRNA (5 µM). Like in the case of E.coli tryptophanyl-tRNA synthetase (1,2) the elution of arginyl-tRNA synthetase from the dye ligand is specific to its cognate tRNA in the presence of Mg^{2+}.

Behaviour of yeast aspartyl-tRNA synthetase

An additional example was examined with aspartyl-tRNA synthetase under similar conditions. This enzyme was not displaced from the Blue-Dextran under the following conditions : ATP (10 mM) or L-aspartic-acid (10 mM) alone in the

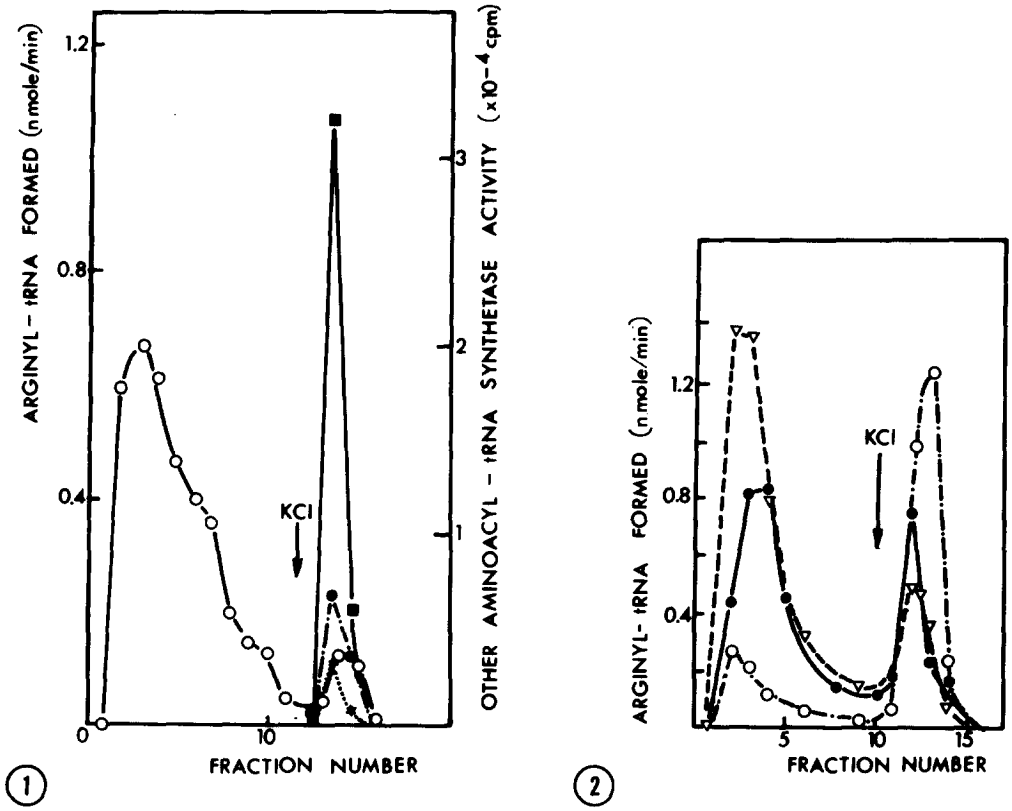

Fig. 1. Specific elution profile of arginyl-tRNA synthetase from the Blue-Dextran-Sepharose by its cognate $tRNA^{Arg}_3$.

About 15 µg of arginyl-tRNA synthetase fraction in 50 mM Tris-HCl buffer (pH 7.5) containing 1 mM DTT, were loaded onto a Blue-Dextran-Sepharose column (0.5 x 2.7 cm). After a washing step with 3 ml of the Tris buffer, the column was eluted with a linear gradient of $tRNA^{Arg}_3$ (0 - 1.2 µM) 50 mM Tris-HCl pH 7.5 which had a total volume of 4 ml. The profile of the gradient was determined by absorbance measurements at 260 nm of the different collected fractions (300 µl). A subsequent elution with KCl 0.5 M was performed. Enzymatic activity was measured as described in "Methods" by addition of 5 µl aliquots to 50 µl reaction mixture and incubation at 37°C for 6 min.

- o——o arginyl-tRNA synthetase ; ■——■ glutamyl-tRNA synthetase

- ·●—·—●·- aspartyl-tRNA synthetase ; ✱·····✱ tyrosyl-tRNA synthetase.

Fig. 2. Comparison of elution profile of arginyl-tRNA synthetase from the Blue-Dextran-Sepharose between cognate $tRNA^{Arg}_3$ and unfractionated yeast tRNA

15 µg of arginyl-tRNA synthetase fraction were loaded onto a Blue-Dextran-Sepharose column (0.5 x 2.7 cm). The enzyme was eluted by a tRNA solution in the Tris buffer, followed by a KCl step (0.5 M) ; fractions of 300 µl were collected and enzymatic activity was measured.

-·o—·o- unfractionated yeast tRNA at 0.1 A_{260} units/ml

—●—●- unfractionated yeast tRNA at 1.0 A_{260} units/ml

--▽--▽-cognate $tRNA^{Arg}_3$ at 0.11 A_{260} units/ml

Table 1 - Comparative degree of dissociation of arginyl-tRNA synthetase from Blue-Dextran-Sepharose by its cognate $tRNA_3^{Arg}$ and other yeast tRNAs.

	Percent arginyl-tRNA synthetase *		
	Elution without $MgCl_2$		Elution with 5 mM $MgCl_2$
	at 0.16 µM tRNA	at 1.6 µM tRNA	at 5 µM tRNA
$tRNA_3^{Arg}$	85	90	65
$tRNA^{Asp}$	30	60	1.5
$tRNA^{Phe}$	27	70	1
$tRNA^{Ser}$	-	30	-
$tRNA_2^{Val}$	12	34	0

* $\frac{\text{tRNA eluate + KCl eluate}}{\text{input activity}}$

15 µg of arginyl-tRNA synthetase fraction in 50 mM Tris-HCl buffer (pH 7.5) containing 1 mM DTT were loaded onto a Blue-Dextran-Sepharose column (0.5 x 2.7 cm). After the washing step with the Tris-HCl buffer, the enzyme was eluted by 3 ml of tRNA solution at the concentration as indicated, followed by a KCl step at 0.5 M in a Tris-HCl buffer. Fractions of 300 µl were collected and enzymatic activity was determined. Previous assays had shown that the arginyl-tRNA synthetase was not affected by tRNA or salt addition in the conditions of enzymatic activity determination.

absence of Mg^{2+} ; ATP (5 mM) + L-aspartic-acid (10 mM) in the presence of 7 mM Mg^{2+}. On the contrary, the enzyme bound on the Blue-Dextran-Sepharose column was readily eluted at 3×10^{-7} M of $tRNA^{Asp}$ when a gradient of $tRNA^{Asp}$ (0 to 2×10^{-6} M) was applied on the column (Fig. 3).

Application to the purification of the arginyl-tRNA synthetase

The high efficiency at low concentration of the cognate $tRNA_3^{Arg}$ to displace, in absence of $MgCl_2$, the arginyl-tRNA synthetase from the Blue-Dextran-Sepharose can be used in the purification of the enzyme.

A preliminary assay with the DEAE enzymatic fraction used in this work (cf. Methods), is reported in Fig. 1 ; the elution by $tRNA_3^{Arg}$ is relatively specific : the contaminating aminoacyl-tRNA synthetase activities tested remained on the column after elution by the cognate $tRNA_3^{Arg}$. Before the Blue-Dextran-Sepharose step, the specific activity of arginyl-tRNA synthetase was 500 units per mg ; after the chromatography, it was about 6500-7000 units per mg compared to a value of 9000 units/mg for the pure enzyme (10).

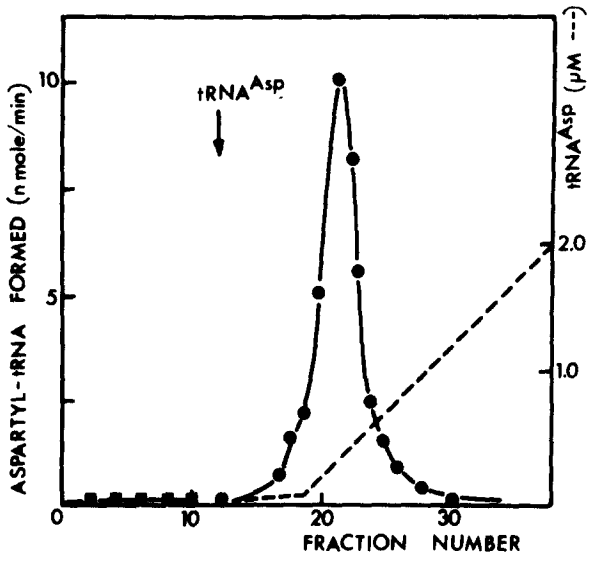

Fig. 3. Elution profile of aspartyl-tRNA synthetase from the Blue-Dextran-Sepharose by its cognate $tRNA^{Asp}$

1.2 mg of DEAE cellulose fraction was loaded on the Blue-Dextran-Sepharose column (0.5 x 2.7 cm). After the washing step, a linear gradient of purified $tRNA^{Asp}$ (0 - 2 μM) in a total volume of 10 ml was applied in the Tris buffer. The profile of gradient and enzymatic activity were measured as described in Fig.1.

DISCUSSION

Yeast arginyl-tRNA synthetase and aspartyl-tRNA synthetase can form a binary complex with Blue-Dextran-Sepharose at low ionic concentration. In the absence of Mg^{2+} the complex can be dissociated by increasing the KCl concentration or by its cognate tRNA or tRNAs being able to form a complex with the enzyme. Moreover, the monomeric substrates, ATP or amino acid individually or together, cannot displace the enzyme from its dye ligand. This is in contrast with the previous results obtained with E.coli tryptophanyl-tRNA synthetase (2) or isoleucyl-tRNA synthetase (3) which showed that ATP dissociates the enzyme from the binary complex when Mg^{2+} is absent. These results suggest that the ATP site is probably not involved directly in the binding of the synthetases studied here onto the Blue-Dextran-Sepharose.

Furthermore, such binding appears to become more specific when the affinity chromatography is performed in the presence of Mg^{2+} which enhances the binding of the aminoacyl-tRNA synthetases to the dye molecules (2,3). Under this condition, not only ATP or ATP plus amino acid has no effect on the dissociation of the enzyme, but tRNAs other than the cognate tRNA can no more displace the

enzyme fixed on Blue-Dextran-Sepharose. It appears consequently that the polypeptide area by which the enzyme is bound to the dye ligand could be located on, or overlapped with the tRNA binding domain.

With regard to the effect of Mg^{2+}, it may play a role in stabilizing the enzyme-dye complex by neutralisation of the negative charges of the blue-dye molecules. An additional effect of the Mg^{2+} in modeling the protein in a suitable conformation to fit in the Blue-Dextran fixation is not excluded.

We have shown that the association constant of arginyl-tRNA synthetase to $tRNA_3^{Arg}$ is dependent from the concentration of Mg^{2+} (K_m = 3.2 x 10^{-7} M at 7 mM Mg^{2+} and 0.6 x 10^{-7} M at 15 mM Mg^{2+} in the presence of 10 mM ATP, Gangloff J., unpublished results). Therefore, one should not expect the thirty times greater concentration of $tRNA^{Arg}$ necessary to displace the enzyme from the Blue-Dextran matrix when Mg^{2+} is added (Table 1). So it seems that Mg^{2+} has a predominant effect in reducing the negative charge groups of the dye molecules as we had already suggested to explain the different effects of ATP on tryptophanyl-tRNA synthetase in the presence of Mg^{2+} and in its absence.

Whatever the explanation, the results showed that the Blue-Dextran-Sepharose can be used successfully in the purification of these enzymes by affinity chromatography.

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