PURIFICATION OF YEAST PHENYLALANYL-tRNA SYNTHETASE BY AFFINITY CHROMATOGRAPHY, ON A $tRNA^{Phe}$-SEPHAROSE COLUMN

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Received 27 July 1972

1. Introduction

The purification of aminoacyl-tRNA synthetases by affinity chromatography may be undertaken using the different ligands of these enzymes: amino acids, aminoacyladenylates or their structural analogues, or tRNA's. Up to now, only techniques using insolubilized tRNA's have bee reported [1, 2]. But none of these techniques has yet allowed the isolation of an aminoacyl-tRNA synthetase in a completely pure state. Contaminating proteins are retained on the columns either by specific interactions with the ligand, or by non specific interactions, for instance ion exchange.

In this paper, we describe the isolation, by an affinity chromatography technique, of yeast phenylalanyl-tRNA synthetase in a completely pure state. Previously this enzyme has been isolated in a homogeneous state by Fasiolo et al. [3] and by Schmidt et al. [4], using conventional techniques. In our method, the chromatographic support was the $tRNA^{Phe}$, which was insolubilized on a hydrazinyl-Sepharose matrix.

2. Experimental and results

2.1. *Materials*

Sepharose 4B was purchased from Pharmacia (Uppsala). Cyanogen bromide came from Schuchardt (München) and hydrazine from Prolabo (Paris). Yeast $tRNA^{Phe}$ was purified from total tRNA (Boehringer, Mannheim) by counter-current distribution, according to Dirheimer et al. [5].

2.2. *Preparation of the chromatographic support*

The Sepharose matrix was modified according to Cuatrecasas [6]. Sepharose 4B was activated with cyanogen bromide at pH 11 (250 mg of BrCN per ml of Sepharose) and was then reacted with hydrazine previously adjusted to pH 10 (2 mmoles per ml of Sepharose). Yeast $tRNA^{Phe}$ was submitted to a periodate oxidation under the following conditions: yeast $tRNA^{Phe}$ 4 mg/ml in 0.1 M acetate buffer pH 5.0, 10 mM $MgCl_2$, 10 mM $NaIO_4$. The tRNA was allowed to react for 1 hr at room temp. The excess of periodate was precipitated by KCl (0.2 M final concentration) during 10 min at 0° and the tRNA was recovered by ethanol precipitation followed by a centrifugation. The precipitate was dissolved in 0.1 M acetate buffer pH 5.0, 10 mM $MgCl_2$ and exhaustively dialyzed against the same buffer.

The oxidized tRNA was allowed to react with the freshly prepared hydrazinyl-Sepharose in the same buffer for 1 hr at 37° and 15 hr at room temp. The modified gel was then washed with 1 M NaCl, in order to elute any tRNA molecules bound by ionic interactions. In these conditions, at least 90% of tRNA was bound to the matrix, as determined by comparing the absorbance of the solution before and after the coupling reaction. This binding is very specific for oxidized tRNA, since only 5% of non oxidized tRNA is bound to the matrix in the same conditions. As the hydrazone bond between tRNA and matrix may be hydrolyzed, thus liberating the tRNA, the chromatographic support was further stabilized by reduction with $NaBH_4$ (3 mg/mg of tRNA) in 0.1 M Tris-HCl buffer pH 8.0, 10 mM $MgCl_2$, during 2 hr at room temp. This treatment decreased the release of tRNA from the column. After this time, the gel was recovered by filtration and rinsed with 0.1 M

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acetate buffer pH 5.5, 10 mM $MgCl_2$.

In these conditions we were able to bind about 10 mg of tRNA per ml of Sepharose at saturation. For chromatographic experiments, we used gels which were charged with only 2.5 to 4 mg of tRNA per ml of gel.

2.3. *Purification of the enzyme*

A crude extract of yeast cells was first partially purified by $(NH_4)_2SO_4$ fractionation. The proteins precipitated between 50 and 70% saturation were recovered and dissolved in the following buffer: 0.05 M acetate or phosphate pH 5.5, 10 mM $MgCl_2$, 0.1 mM EDTA, 5 mM β-mercaptoethanol, 10% glycerol. These conditions are optimal for the formation of a complex between $tRNA^{Phe}$ and phenylalanyl-tRNA synthetase, as found by Befort et al. [7]. The resulting solution was filtered on a G-25 Sephadex column, equilibrated with the same buffer, to eliminate the residual ammonium sulfate. The fractions containing the enzymic activity were pooled and applied to the tRNA column. The elution was performed with the same buffer. The large bulk of the proteins emerged from the column as a single peak, almost completely deprived of phenylalanyl-tRNA synthetase activity. After rinsing, the elution buffer was changed for a 0.1 M Tris-HCl buffer pH 8.0, 0.2 M KCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 5 mM β-mercaptoethanol, 10% glycerol. In these conditions, the complex between $tRNA^{Phe}$ and phenylalanyl-tRNA synthetase was dissociated and the enzyme emerged from the column (fig. 1). The fractions containing activity were pooled and concentrated by vacuum dialysis. The recovered proteins were analysed by polyacrylamide gel electrophoresis in the conditions described by Jovin et al. [8]. Fig. 2 shows the scanning of the gel. As can be seen, phenylalanyl-tRNA synthetase amounts to about 5% of total proteins. Three contaminating proteins can be detected, which have not been, up to now, identified.

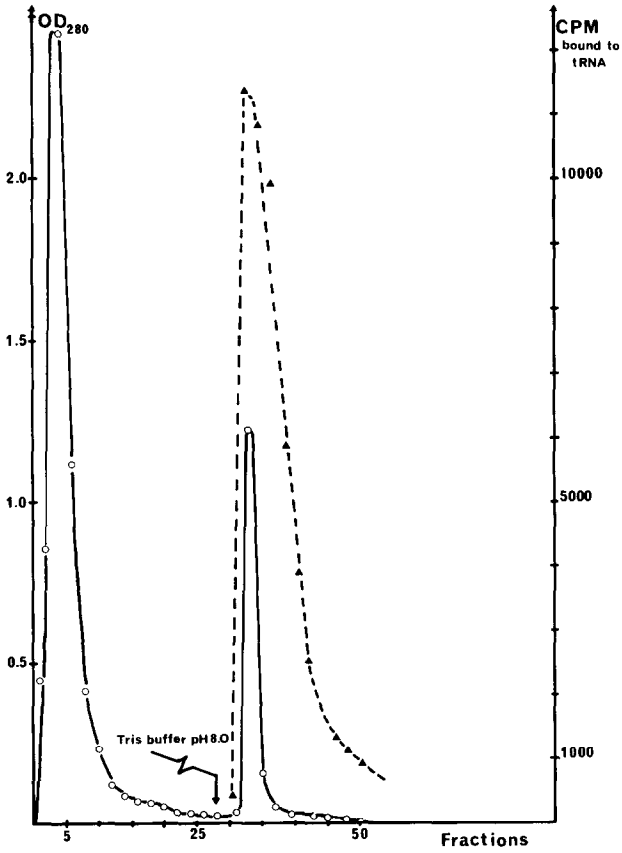

Fig. 1. Elution pattern of the $tRNA^{Phe}$-Sepharose column: (o—o—o) optical density; (▲- -▲- -▲) activity.

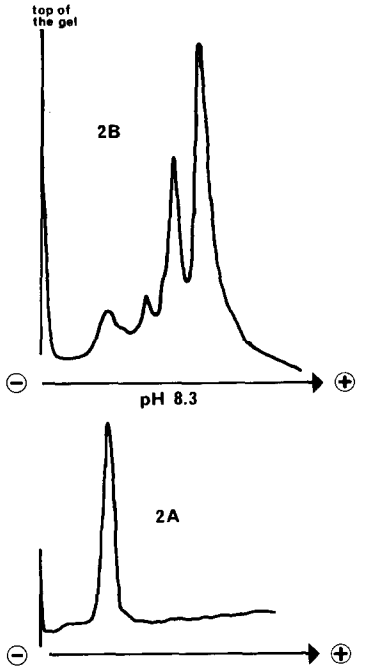

Fig. 2. Scanning of polyacrylamide gel electrophoregrams of proteins recovered from $tRNA^{Phe}$-Sepharose column: 2 A) Standard phenylalanyl-tRNA synthetase prepared by conventional techniques. 2 B) Proteins recovered from $tRNA^{Phe}$-Sepharose column.

The small number of contaminants led us to infer that these proteins were retained by specific interactions, rather than by ion exchange. The specificity of the interactions could be of different orders:

i) Several proteins could have recognized specifically the $tRNA^{Phe}$ (for instance the phenylalanyl-tRNA synthetase or the enzyme responsible for the Y base modification).

ii) Other proteins could have recognized general tRNA features (for instance the methylases or tRNA-ATP-CTP nucleotidyltransferase).

iii) Finally, the interaction might have been specific for a general RNA character (for instance the RNAases).

It should be possible to eliminate proteins belonging to the last two groups by first filtering the enzymic extract on a column charged with tRNA's lacking $tRNA^{Phe}$.

Two columns were thus prepared, the first one (diameter 5 cm, height 4 cm) charged with 330 mg of purified tRNA lacking $tRNA^{Phe}$ and the second one (diameter 5 cm, height 2 cm) with 125 mg of purified $tRNA^{Phe}$. A crude extract resulting from the grinding of 140 g of yeast in 70 ml of 0.1 M Tris-HCl buffer pH 8.0, was fractionated by $(NH_4)_2SO_4$ precipitation between 50 and 70% of saturation. This fraction was dissolved in the minimum volume of 0.1 M acetate buffer pH 5.5, 10 mM $MgCl_2$, 0.1 mM EDTA, 5 mM β-mercaptoethanol, 10% glycerol and filtered on a G-25 Sephadex column (30 × 1.5 cm), equilibrated in the same buffer. The fractions containing activity were pooled (120 ml, 5 mg proteins/ml) and passed through the two columns. The flow rate was 60 ml/hr. After washing with the same buffer, the columns were separated and the second one (charged with $tRNA^{Phe}$) was eluted, first with a 0.1 M phosphate buffer pH 6.5, 10 mM $MgCl_2$, 0.1 mM EDTA, 5 mM β-mercaptoethanol, 10% glycerol, and then with a 0.1 M Tris-HCl buffer pH 8.0, 0.2 M KCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 5 mM β-mercaptoethanol, 10% glycerol. All the experiments were performed at 4°. The elution pattern is shown on the fig. 3. Fractions containing activity were pooled and concentrated by vacuum dialysis; glycerol was added to a final conc. of 50%. Fig. 4 shows the electrophoretic analysis, on polyacrylamide gel, in the conditions described above. As can be seen the enzyme is homogeneous. Furthermore, no detectable charging activity for other amino acids

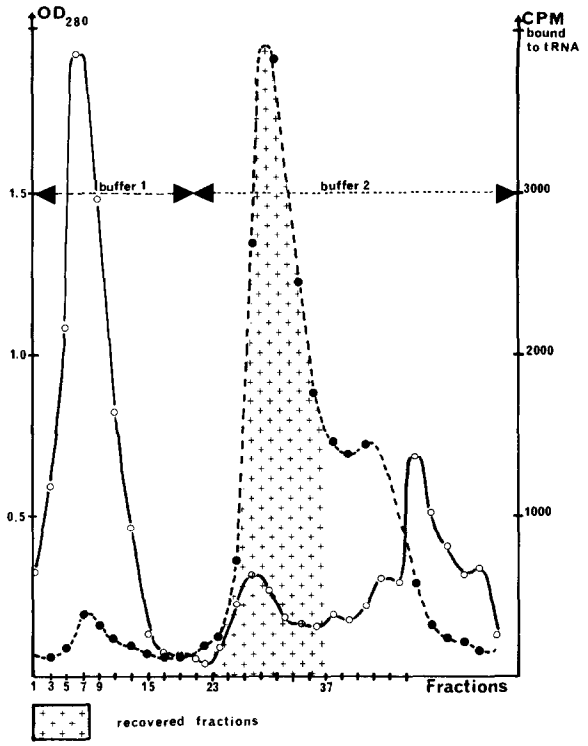

Fig. 3. Elution pattern of the $tRNA^{Phe}$-Sepharose column after filtration of the enzymic extract on a Sepharose column charged with tRNA's lacking $tRNA^{Phe}$: (○—○—○) optical density; (●--●--●) activity. Buffer 1: 0.1 M phosphate pH 6.5, 10 mM $MgCl_2$, 0.1 mM EDTA, 5 mM β-mercaptoethanol, 10% glycerol. Buffer 2: 0.1 M Tris-HCl pH 8.0, 0.2 M KCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 5 mM β-mercaptoethanol, 10% glycerol.

was observed. The specific activity for phenylalanine is around 1,800 to 2,000 units per mg, which is in good agreement with those described by the other authors, using conventional techniques [3, 4]. A typical purification is summarized in table 1.

3. Discussion and conclusion

Our results clearly show that it is possible to purify aminoacyl-tRNA synthetase by affinity chromatography, the cognate tRNA being used as the ligand. Several authors, using a similar approach, were unsuccessful in obtaining completely pure aminoacyl-tRNA synthetases. Nelidova et al. [1], using oxidized tRNA's, bound to a polyacrylhydrazid-agar gel, re-

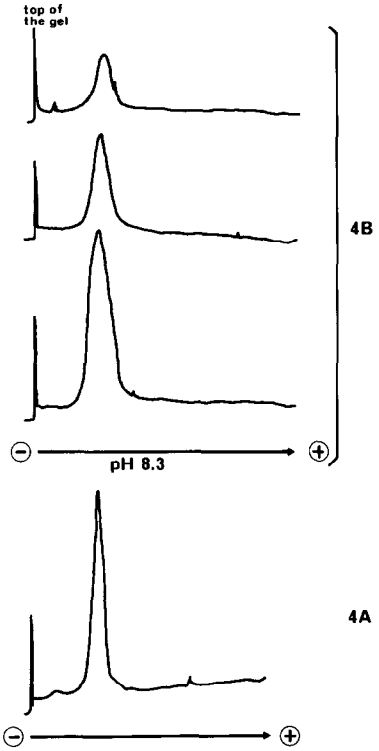

Fig. 4. Scanning of polyacrylamide gel electrophoregrams of phenylalanyl-tRNA synthetase recovered from $tRNA^{Phe}$-Sepharose column, after filtration of the enzymic extract on a Sepharose column charged with tRNA's lacking $tRNA^{Phe}$. 4 A) Standard phenylalanyl-tRNA synthetase. 4 B) Phenylalanyl-tRNA synthetase recovered from $tRNA^{Phe}$-Sepharose column.

ported enrichment factors, for rat liver valyl- and lysyl-tRNA synthetases, ranging from 30 to 90-fold. Bartkowiak et al. [2], using isoleucyl-$tRNA^{Ile}$ bound to bromoacetamidobutyl-Sepharose, reported an enrichment factor of 27.5 for *E. coli* isoleucyl-tRNA synthetase. The reason for this incomplete purification is chiefly the fact that other proteins are retained on a column charged with a specific tRNA. Our results demonstrate that the interactions which take place between these contaminating proteins and the chromatographic support are less specific since they are retained on a column charged with tRNA's lacking $tRNA^{Phe}$. Using this column in conjunction with the $tRNA^{Phe}$ column, we were able to isolate a pure phenylalanyl-tRNA synthetase.

tRNA being a rather labile material, it is advisable to filter over the columns a partially purified enzyme, so as to eliminate, as completely as possible, the ribonucleases, which could hydrolyse the bound tRNA, although the acidic pH which is used for the binding of the enzyme is far from the optimal pH of these nucleases. We have used the same column for three purifications, without any detectable alteration of the chromatographic support.

Table 1
Purification of the enzyme.

Step	Enzyme recovered (units)	Yield (%)
Crude extract	17,600	100
$(NH_4)_2SO_4$ fraction (50 to 70% saturation)	11,900	67
Washes of the two columns	1,460	
Elution of the first column	300	
Elution of the second column		
a) first peak	300	
b) second peak	5,700	35

The described purification is much more rapid than the conventional purifications previously described [3, 4] and it affords a larger overall yield. Its drawback is to require the preliminary purification of the cognate tRNA. We are now trying to develop a general method, which would not require the purification of a specific tRNA.

Acknowledgements

This investigation was supported by the C.N.R.S. (L.A. 119), the Délégation Générale à la Recherche Scientifique et Technique (Action concertée Interactions Moléculaires en Biologie) and by the Commissariat a l'Energie Atomique.

We wish to thank Dr. Dirheimer for his kind gift of purified yeast $tRNA^{Phe}$ and Mrs. Monique Schlegel and Nelly Menard for their skilful technical assistance.

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