BEHAVIOUR OF PHENYLALANYL-tRNA FROM *HALOBACTERIUM CUTIRUBRUM* ON *BD-CELLULOSE

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1. Introduction

Transfer RNAs charged with aromatic amino acids have been reported to bind firmly to BD-cellulose, a property which has been used for the purification of $tRNA^{Trp}$ and $tRNA^{Tyr}$ of yeast [1]. These aminoacyl-tRNAs are eluted only with a salt solution containing ethanol or an organic compound which disrupts hydrophobic interactions (as well as hydrogen bonding); such a property allows them to be separated from uncharged tRNAs which elute with salt solution alone. This affinity for BD-cellulose has been attributed to the interaction of the aromatic amino acid on the tRNA with the benzoyl groups on the matrix. Individual charged isoaccepting species of tRNA have not been separated from each other by this method and, since each will have an aromatic amino acid attached, it has been assumed that they behave in an identical manner [1, 2]. Maxwell et al. [1] have proposed that it should be possible to isolate $tRNA^{Trp}$, $tRNA^{Tyr}$ and $tRNA^{Phe}$ from the tRNA of any organism using this method provided that the uncharged tRNA can be eluted by a salt solution.

In order to extend our studies on the mechanism of protein synthesis in the extremely halophilic bacterium *Halobacterium cutirubrum*, [3–5] the purification of phenylalanine specific tRNA was undertaken. This report describes the behaviour of charged isoaccepting species of $tRNA^{Phe}$ on BD-cellulose. In contrast to results obtained with aromatic aminoacyl tRNAs from other organisms, charged halophile isoaccepting $tRNA^{Phe}$ species behave quite differently. One species binds firmly to BD-cellulose and requires ethanolic salt solution for its removal, while another is eluted with salt solution alone, even though it is charged with an aromatic amino acid. Thus, the properties of the tRNA itself are as important as the nature of the amino acid in determining whether or not charged tRNA will bind firmly to BD-cellulose.

2. Materials and methods

The preparation of the aminoacyl-tRNA synthetases (pH 5 fraction) and unfractionated tRNA from *H. cutirubrum* was as described previously [5], except that the final ethanol precipitate of tRNA was dissolved in water, dialyzed against glass distilled water and finally freeze dried; it was stored in a desiccator at −20°. The tRNA was charged with ^{14}C-phenylalanine in a large scale incubation mixture increased proportionally from that described by Griffiths and Bayley [5]; the reaction mixture contained both labelled and unlabelled phenylalanine to give a final concentration of 20–60 nmoles/ml and a final specific activity between 10 and 40 mCi/mmole; charging was carried out at an RNA concentration of 20–50 A_{260} units/ml. After incubation at 37° for 25 min, the reaction was stopped by adding cold 1 M sodium acetate (pH 4.5), to give a final concentration of 0.1 M, and the mixture dialyzed for 5 hr, at 4°, against 0.01 M sodium acetate (pH 4.5). Precipitated protein was removed by centrifugation and the mixture then

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* *Abbreviation:* Benzoylated Diethylaminoethylcellulose: BD-Cellulose.

shaken with an equal volume of phenol (water saturated) for 10 min. RNA was precipitated from the aqueous phase by adding 2 M NaCl, to give 0.2 M solution, together with 2 vols. of ice cold ethanol. After standing at −20° for 3 hr, the precipitate was collected, redissolved in 1 M sodium acetate (pH 4.5) and reprecipitated with ethanol as before. The final precipitate was dissolved in the required volume of 0.3 M NaCl, 0.01 M $MgCl_2$ and 0.01 M Na acetate (pH 4.5).

BD-cellulose (Schwartz Bioresearch, 20–50 mesh) columns were packed as described by Gillam et al. [6]. Solutions for developing the columns each contained 0.01 M $MgCl_2$ and 0.01 M Na acetate (pH 4.5) together with 0.3 M NaCl, or 1.0 M NaCl or 1.0 M NaCl-20% ethanol (v/v) as indicated in the diagrams. All the columns were developed at 4°. To measure the radioactivity in fractions from the column, 1 ml samples of the fractions were each mixed with 1 ml of cold water, 0.1 ml of a solution of serum albumin (5 mg/ml), to act as carrier, and 2 ml cold aqueous 10% trichloroacetic acid (w/v). After standing in ice for 10–15 min, the precipitated material was washed once with 4 ml of cold 5% trichloroacetic acid by centrifugation, collected on a Millipore filter (0.45 μm), washed again with 3 ml of 5% trichloroacetic acid, dried in an oven for 20 min at 65° and assayed for radioactivity in a liquid scintillation counter as described previously [3]. Estimation of the phenylalanine acceptor activity of the tRNA was performed in concentrated salt solution by the method of Griffiths and Bayley [5].

3. Results and discussion

The chromatographic behaviour of halophilic tRNA on a BD-cellulose column, developed by stepwise elution, is shown in fig. 1. The bulk of the tRNA, the salt fraction, was eluted from the column with a solution containing 1.0 M NaCl. The remaining material, the ethanol fraction, was removed by the addition of ethanol to the eluting system. Total recovery of absorbance from the column was about 90%, with 98% of the recovered phenylalanine acceptor activity being found in the combined salt fraction eluate. In this respect, the behaviour of halophile $tRNA^{Phe}$ resembles that of *Escherichia coli* [7] and differs from that of yeast and rat liver [7, 8] which bind tightly to BD-cellulose in the uncharged form and are recovered from the ethanol fraction. The modified nucleoside Y, which appears to be responsible for the unusual binding behaviour of uncharged yeast and rat liver phenylalanine tRNA [6, 8, 9]] is, therefore, unlikely to be present in $tRNA^{Phe}$ of *H. cutirubrum*. Fig. 2 shows the absorbances at 260 nm and the radioactivity in the fractions found on repeating the chromatography of the salt fraction after charging with ^{14}C-phenylalanine. About 50% of the ^{14}C-phenylalanyl-tRNA eluted with 1.0 M NaCl in the form of a broad peak with some tailing; the other 50% was eluted from the column as a sharp peak with 1.0 M NaCl-20% ethanol. This elution pattern suggests the presence of at least two species of $tRNA^{Phe}$ in *H.cutirubrum*. One of these is bound quite firmly to BD-cellulose when esterified with phenylalanine and requires ethanolic salt solution for its elution. The other, even though aminoacylated, is weakly bound and is eluted with salt solution alone.

That this result was not due to a nonspecific leakage as described by Maxwell et al. [1], but was indeed due to one or more species of charged $tRNA^{Phe}$ which had a low affinity for BD-cellulose was shown when the salt eluted ^{14}C-phenylalanyl-tRNA was rechromatographed. The material eluted with 1.0 M NaCl (fig. 2) was deacylated by incubation at 37° for 40 min in 2 M tris-HCl buffer, pH 8, isolated and recharged with ^{14}C-phenylalanine. On rechromatography, 75% of the ^{14}C-phenylalanyl-tRNA eluted from the column with 1.0 M NaCl solution alone and only 25% required ethanol for its elution (fig. 3; compare fig. 2). This remaining ethanol-eluted material was probably due to tRNA which had not been charged during the first aminoacylation reaction.

Two points emerge from this work. Firstly, tRNAs charged with aromatic amino acids do not all bind firmly to BD-cellulose; secondly, charged iso-accepting species can behave differently*. Thus, the properties of the tRNA itself are as important as the nature of the amino acid in determining whether or not charged tRNA binds firmly to BD-cellulose. Increased binding cannot, therefore, be attributed solely to the

* While this work was in progress, Henes et al. [10] reported that phenoxyacetyl derivatives of the two methionyl tRNAs of *Escherichia coli* also behaved differently on BD-cellulose when chromatography was carried out at 4°.

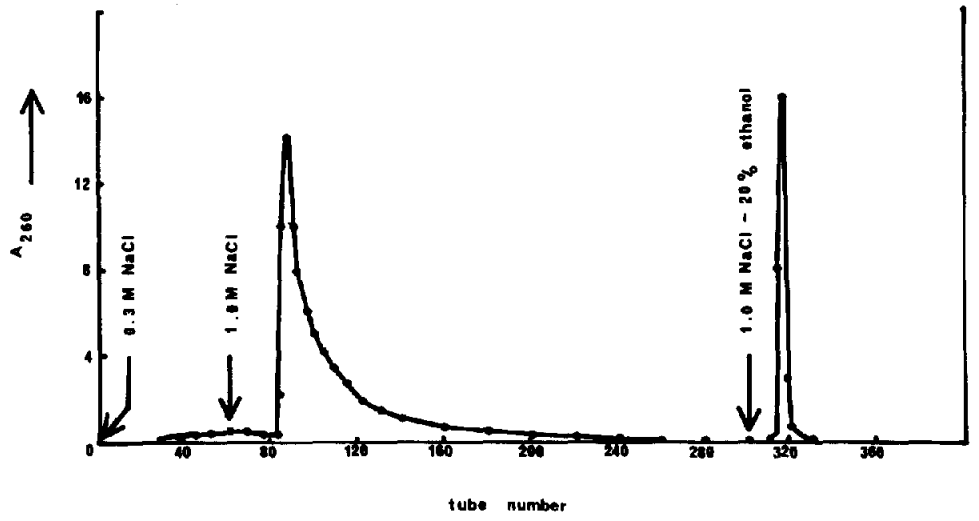

Fig. 1. Chromatography of tRNA (2600 A_{260} units) on a BD-cellulose column (2.5 X 36 cm). Fractions (10 ml) were collected at a flow rate of 40 ml/hr. Elution was stepwise as indicated.

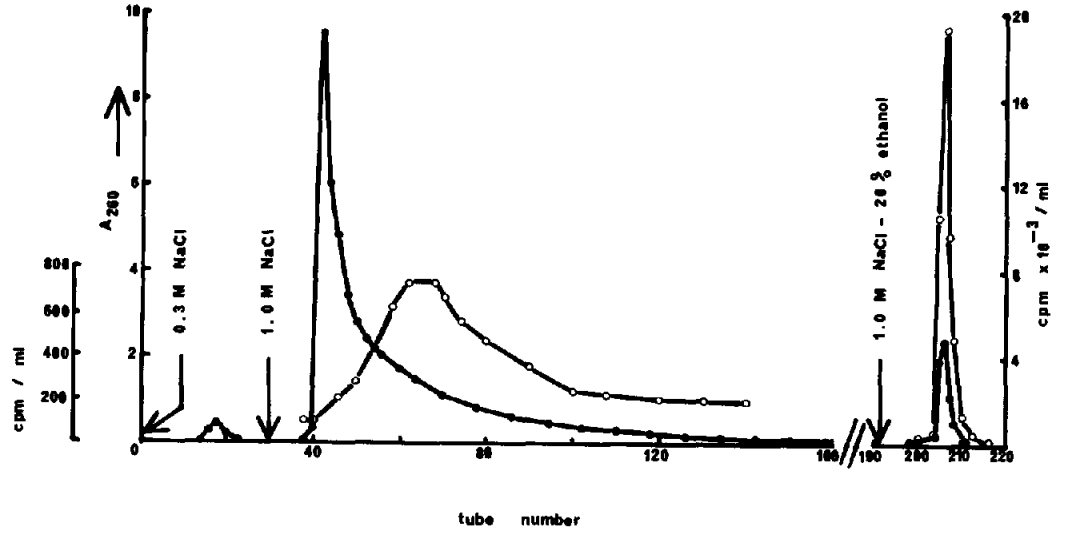

Fig. 2. Chromatography of the salt fraction tRNA (1500 A_{260} units) charged with ^{14}C-phenylalanine (40 mCi/mmole) on a BD-cellulose column (2.5 X 36 cm). Elution was stepwise with 10 ml fractions being collected at a flow rate of 40 ml/hr. Absorbance at 260 nm ●—●; radioactivity: ○—○.

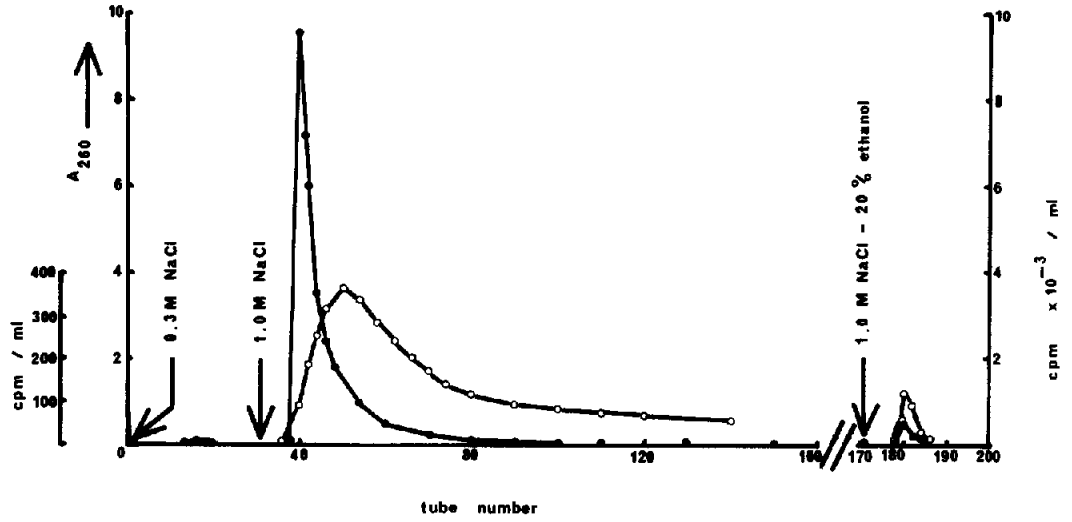

Fig. 3. Rechromatography of the tRNA (340 A_{260} units) eluted with 1.0 M NaCl in fig. 2, after deacylation and recharging with ^{14}C-phenylalanine (36 mCi/mmole), on a BD-cellulose column (1.5 X 26 cm). Fractions (5 ml) were collected at a flow rate of 40 ml/hr using stepwise elution. Absorbance at 260 nm: •—•; radioactivity: ○—○.

presence of an aromatic amino acid on the tRNA. Some other factor, depending either on the base composition of the tRNA or on its configuration, also appears to be involved.

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