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Note

Application of spermine–Sepharose column chromatography to the separation of plant-specific transfer ribonucleic acids and aminoacyl-tRNA synthetases

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Substantial amounts of single species of transfer ribonucleic acids (tRNAs) and aminoacyl-tRNA synthetases (AARS) are needed to carry out studies on chemical modifications, crystallization, etc., with these macromolecules[1]. Published methods for purification of tRNA and AARS include: partition chromatography, ion-exchange chromatography on diethylaminoethyl cellulose (DEAE-cellulose), DEAE-Sephadex, hydroxyapatite, phosphocellulose or benzoylated DEAE-cellulose (BD-cellulose); and chemical methods involving modification of tRNA[2]. Also, techniques of affinity or hydrophobic chromatography have given good results in the purification of many enzymes[3] and nucleic acids[4].

Fractionation of nucleic acids, (*e.g.*, RNA), and proteins is based on several factors such as chain length, degree of interaction with the matrix, charge of nucleic bases or amino acids, hydrophobicity of the constituents, but the precise mechanism of separation is not fully understood[5].

In the course of our studies on plant tRNAs and aminoacyl-tRNA synthetases we have checked several previously tested techniques and frequently felt the need for a new method of general applicability, based on another separation principle.

MATERIALS AND METHODS

Unfractionated tRNA from yellow lupin seeds was obtained as described previously[6]. Protein extract from lupin seeds after fractionation in 45–65% of ammonium sulphate was prepared as in ref. 7, CNBr-activated Sepharose 4B was purchased from Pharmacia (Uppsala, Sweden). Spermine was from Serva (Heidelberg, G.F.R.). Other reagents were reagent grade. Spermine–Sepharose was prepared from 5 g of CNBr-activated Sepharose 4B and from 5 g of spermine essentially as described[8].

RESULTS AND DISCUSSION

It is well known that some naturally occurring diamines and polyamines interact specifically with nucleic acids and enzymes[9]. The exact nature of these interactions is not yet understood[9]. The affinity of some polyamines for tRNA is utilized in the

preparation of crystals of tRNA for X-ray crystallographic studies. Addition of spermine to the tRNA solution influences the type of crystal obtained and is essential for the preparation of certain crystal forms[10].

The aim of this work was to use this type of interaction to separate individual species of tRNAs and AARS. For these studies we prepared a spermine–Sepharose matrix. Spermine is among the longest of naturally occurring polyamines. It is a linear molecule, NH_3^+–$(CH_2)_3$–NH_2^+–$(CH_2)_4$–NH_2^+–$(CH_2)_3$–NH_3^+, with four positively charged nitrogen atoms. When fully extended it is over 15 Å long[11]. We have coupled this amine directly, to the matrix of Sepharose without any spacer.

A mixture of the specific tRNAs from yellow lupin seeds was fractionated on a spermine–sepharose column (Fig. 1). The specific activity for tRNA was tested in an aminoacylation reaction[6]. It is seen that $tRNA^{Arg}$ and $tRNA^{Phe}$ are eluted later from the column, but $tRNA^{Met}$ is eluted very early in a NaCl gradient. Specific activities of $tRNA^{Met}$, $tRNA^{Arg}$ and $tRNA^{Phe}$ in an unfractionated preparation were 25, 48 and 67 pmoles of amino acid per A_{260} unit (pM/A_{260}), respectively. After column chromatography on a spermine–Sepharose column the specific activities approach values of 100 pM/A_{260} for $tRNA^{Met}$, 150 pM/A_{260} for $tRNA^{Phe}$ and 220 pM/A_{260} for $tRNA^{Arg}$. If we consider $tRNA^{Arg}$, for example, it is possible to get good purification if the BD-cellulose column, where $tRNA^{Arg}$ is eluted in the middle of the chromatogram, is followed by a spermine–Sepharose column, where the order of elution is different (Fig. 1).

The high fractionation capability for individual specific tRNAs is shown on

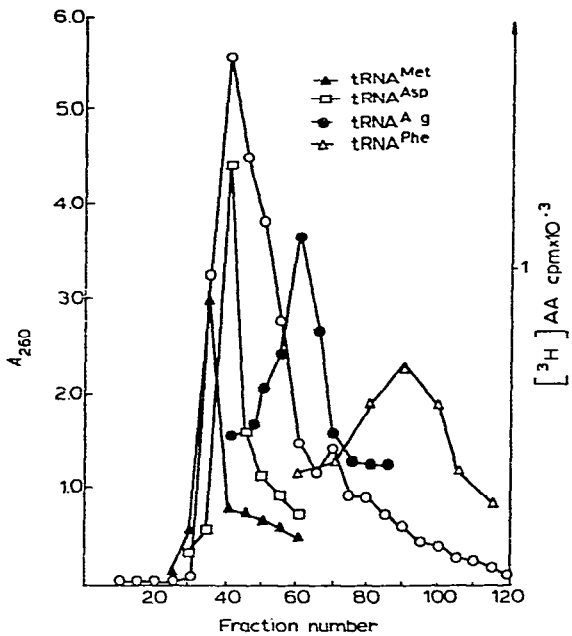

Fig. 1. Chromatography of unfractionated yellow lupin tRNA on spermine–Sepharose column (25 × 0.9 cm). The column was equilibrated with 0.01 *M* sodium acetate buffer, pH 4.5, containing 0.01 *M* $MgCl_2$. 760 A_{260} units of tRNA were applied to the column and eluted with 2 × 500 ml of gradient 0.0–0.6 *M* NaCl in the above buffer. Fractions of 5.2 ml were collected every 8 min. ○, A_{260}.

Fig. 2. Fractions containing aspartic acid and valine specific tRNAs after BD-cellulose column chromatography were collected and applied to the spermine–Sepharose column. One can see (Fig. 2) the very good separation of isoacceptors $tRNA^{Asp}$ and $tRNA^{Val}$. The specific activities of these tRNAs were 5–7 times higher than on the previous column.

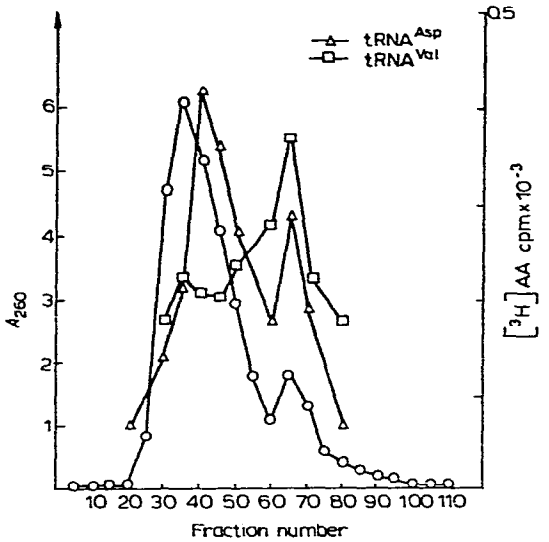

Fig. 2. Chromatography of partially purified $tRNA^{Asp}$ and $tRNA^{Val}$ (from BD-cellulose column) on spermine–Sepharose column (25 × 0.9 cm). Conditions of separation as in Fig. 1. ○, A_{260}.

From these results, the question immediately arose as to the reason for the differentiation of specific tRNAs on the column. It is clear that the main part of the tRNA interaction with the column is of an ionic nature. But it is also interesting to determine the influence of other factors on the resolution power of the column. It is known that both spermine and magnesium ions are competent in the synthesis of many aminoacyl-tRNAs and other ribosomal reactions[9,12]. Therefore, we fractionated total yellow lupin tRNA on a spermine-Sepharose column in a magnesium ion concentration gradient. A very interesting picture appeared (Fig. 3). Especially pronounced results are noted for $tRNA^{Arg}$ and $tRNA^{Phe}$ where isoacceptors of these tRNAs can be separated. From this experiment one can conclude that besides general ionic interaction of spermine and tRNA there is also specific binding due to the above mentioned affinity of spermine and tRNA. It was demonstrated previously that one molecule of tRNA binds six molecules of spermine in solution[13]. Also, the binding of polyamines to tRNA is preferential to that of magnesium, suggesting that polyamine–tRNA complexes could exist *in vivo*[14]. Crystallographic studies of $tRNA^{Phe}$ from yeast showed that the two spermine molecules are tightly bound and are important in maintaining the overall folding of the tRNA molecule[11].

If specific interaction between spermine and tRNA takes place on the column, then specific tRNAs can be eluted from the column with linearly increasing concentrations of magnesium ions. Depending on the strength of this binding, it is possible to resolve individual tRNAs and even tRNA isoacceptors (Fig. 3). A similar approach

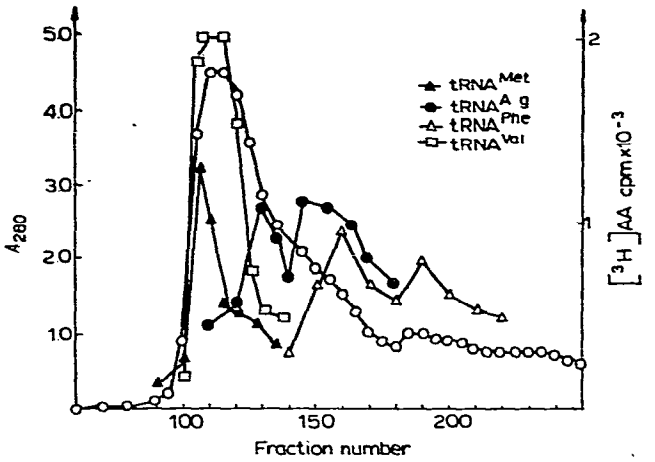

Fig. 3. Spermine–Sepharose column (25 × 0.9 cm) chromatography of total yellow lupin tRNA. The column was equilibrated with 0.01 *M* sodium acetate buffer, pH 4.5. 760 A_{260} units of tRNA were applied to the column and eluted with 2 × 500 ml gradient of 0.0–0.2 *M* $MgCl_2$ in the above buffer. Fractions of 1.9 ml were collected at a flow-rate of 22.8 ml/h. ○, A_{280}.

was used by Seibert and Zahn[15], who prepated spermine–Sepharose from spermine and CH-Sepharose 4B for separation of 4S, 5S, 16S and 23S of *Escherichia coli* RNA. They obtained RNA species of high purity. The difference between their method and ours is the preparation of the matrix. They used Sepharose with a six-carbon spacer group[15]. Some authors have concluded that the presence of a spacer facilitates the interaction between high-molecular-weight RNA and the matrix[8].

From our studies one can conclude that direct linkage between spermine and Sepharose did not influence the interaction of tRNA with the immobilized spermine. We would like to add that chromatography of total tRNA on unmodified Sepharose, under the conditions applied here (Figs. 1 and 2), yields a sharp peak at void volume of the column. At high concentrations of ammonium sulphate, Sepharose 4B fractionated tRNA very efficiently[16].

To test the capacity of the spermine–Sepharose column for the purification of enzymes, we have chromatographed a crude protein extract of enzymes from yellow lupin seeds (Fig. 4). It is seen from Fig. 4 that aminoacyl-tRNA synthetases specific for methionine (MetRS), arginine (ArgRS) and phenylalanine (PheRS) are slightly retained on the column, but their activity was low. Some of them are eluted in the void volume because of the low column capacity to bind the enzymes.

We have also applied this column for the final purification of MetRS after the hydroxyapatite column (Fig. 5). Nearly all MetRS activity is eluted in the void volume. Additional peaks of the enzyme were observed. These are probably products of the aggregation of pure synthetase which we have observed previously on polyacrylamide gel electrophoresis of concentrated yellow lupin seed MetRS[17]. Similar phenomena have been noted for LeuRS[18].

Finally, we would like to emphasize that spermine–Sepharose column chromatography offers many possibilities for purification of all tRNAs and aminoacyl-tRNA synthetases. It is simple and rapid.

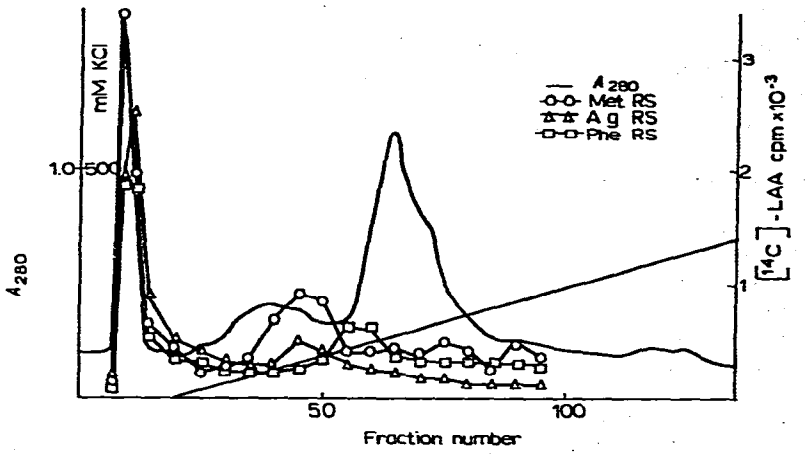

Fig. 4. Chromatography of crude protein extract from yellow lupin seeds on spermine–Sepharose column (25 × 0.9 cm). Protein extract, after fractionation with 45–65% of ammonium sulphate and desalting on Sephadex G-25, was applied to the column. The buffer used was 50 m*M* Tris–HCl, pH 7.1, 10 m*M* $MgCl_2$, 10 m*M* KCl, 10 m*M* 2-mercaptoethanol and 10% glycerol. The column was eluted with a gradient of 10–400 m*M* KCl in the above buffer; total gradient volume, 400 ml. Fractions of 3 ml were collected at a flow-rate of 18 ml/h.

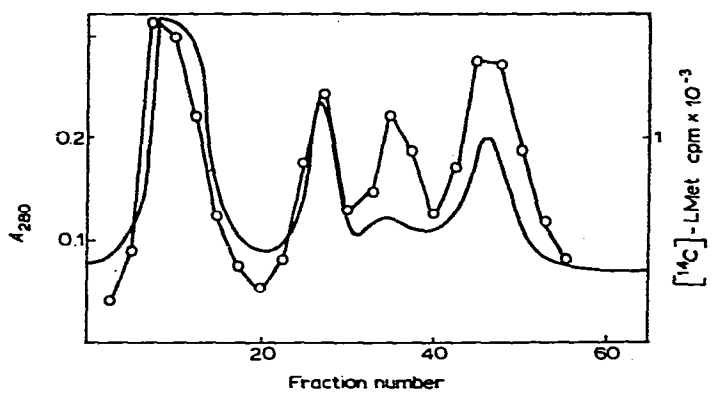

Fig. 5. Spermine–Sepharose column (25 × 0.9 cm) chromatography of methionyl-tRNA synthetase after hydroxyapatite column. An 18-mg amount of MetRS was applied and eluted with 250 ml of 10–250 m*M* KCl gradient in buffer (as in Fig. 4). Fractions of 2.5 ml were collected at a flow-rate of 40 ml/h. —, A_{280}; O—O, Met RS activity.

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