Journal of Chromatography, 206 (1981) 600–605
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,432

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Heparin–Sepharose column chromatography as a new method for the purification of aminoacyl-tRNA synthetases

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(Received September 25th, 1980)

Although various methods exist for the purification of plant aminoacyl-tRNA synthetases (AARS, E.C. 6.1.1.–), many efforts have been made to find new, more efficient techniques which are also easier and simpler. The most promising methods are based on the principle of affinity chromatography[1]. AARS substrates have been immobilized on a support and then used to specifically retain the enzyme. Immobilized tRNA[2] and amino acid derivatives[3–6] have successfully been used to purify aminoacyl-tRNA synthetases.

In this paper we describe for the first time the application of heparin–Sepharose CL 6B for the separation of plant aminoacyl-tRNA synthetases. The method is not only rapid but also gives reproducible preparations of the enzymes with high specificity.

MATERIALS AND METHODS

A protein mixture was extracted from 250 g of milled yellow lupin seeds, essentially as described previously[7]. The aminoacyl-tRNA synthetases were fractionated with ammonium sulphate (35–65%) at 0°C. After dialysis against buffer C, the protein extract was passed through DEAE-cellulose (DE-23)[8] and then applied to a Sephadex G-150 or heparin–Sepharose CL 6B column. Phenylalanine and arginine specific tRNAs from yellow lupin seeds were prepared in our laboratory[9,10].

The activity of aminoacyl-tRNA synthetases was tested as described previously[7]. The inhibition of the charging tRNA by heparin (Calbiochem, Los Angeles, CA, U.S.A.) was tested under the conditions described[7]. The activity of elongation factor 1 (EF 1) was tested under the conditions described in ref. 11.

DEAE-Sephadex A-50, Sephadex G-150 and heparin–Sepharose CL 6B were purchased from Pharmacia (Uppsala, Sweden). ^{14}C-Labelled amino acids were obtained from the Radiochemical Centre (Amersham, Great Britain). The protein concentration was measured by the method of Bradford[12].

Buffers: A, 50 m*M* Tris–HCl, pH 7.1, 10 m*M* KCl, 10 m*M* $MgCl_2$, 10 m*M* 2-mercaptoethanol, 10% glycerol; B, 50 m*M* Tris–HCl, pH 7.1, 10 m*M* KCl, 10 m*M* $MgCl_2$, 10 m*M* 2-mercaptoethanol, 20% glycerol; C, as buffer B, but 180 m*M* KCl;

0021-9673/81/0000–0000/$02.50

D, 25 m*M* Tris–HCl, pH 7.6, 25 m*M* KCl, 25 m*M* $MgCl_2$, 6 m*M* 2-mercaptoethanol, 2 m*M* EDTA, 20% glycerol.

RESULTS AND DISCUSSION

A large number of methods for the purification of specific enzyme groups has been described. For example, classical ion-exchange column chromatography[13], hydrophobic or affinity chromatography with immobilized substrates have been applied to AARS[14–17]. The last two methods have sometimes yielded satisfactory results. Interesting in this respect is the affinity elution of aminoacyl-tRNA synthetases with a specific substrate[14]: the crude yeast enzymes are applied to an ion-exchanger (*e.g.*, phosphocellulose) and then eluted with a specific tRNA. With this method pure AARS are obtained very rapidly.

In a previous paper[18] we described the simultaneous purification of methionyl- (MetRS), phenylalanyl- (PheRS) and arginyl- (ArgRS) tRNA synthetases using column chromatography on Sephadex G-150, DEAE-cellulose and DEAE-Sephadex A-50. This method gave homogeneous enzymes with a specific activity of about 50–150 units (one unit corresponds to the charging of 1 nmol of amino acid to tRNA per mg of protein at 37°C in 1 min). This activity was, however, slighty lower than observed for the enzyme of *Escherichia coli* or yeast[19].

We have found that some AARS isolated from yellow lupin seeds are tightly bound to the column during chromatography on concanavalin–Sepharose 4B (Con A–Sepharose). It can be concluded that these preparations contained saccharides[8]. Sugar analyses showed the presence of glycosides which specifically interacted with concanavalin A[8]. Similar saccharides have been found in lysyl-tRNA synthetase and/or arginyl-tRNA synthetase from rat liver[20]. These results suggest the existence of specific interactions between certain sugar moieties and proteins. If this is true, it should be possible to differentiate the AARS during column chromatography in which a sugar residue is coupled to the matrix. For this purpose we used heparin–Sepharose column chromatography[21]. The heparin in heparin–Sepharose CL 6B occurs naturally in mammals; this mucopolysaccharide is an unbranched, highly sulphated glucosaminoglycan which consists mainly of repeating disaccharide sequences of α-L-indopyranuronic acid-2-sulphate and 2-deoxy-2-sulphamino-α-D-glucopyranose-6-sulphate linked through positions 1 and 4[22]. The main characteristic of heparin is that it contains a large number of amino groups combined with sulphate groups, the latter being quite labile in acidic media. It also contains small quantities of other sugars such as galactose or xylose[23]. Owing to its polyanionic nature, heparin interacts with many cationic biological compounds, and such interactions may be used as the basis for purification by affinity chromatography on heparin–Sepharose[21].

To test the ability of the heparin–Sepharose column to purify plant AARS, a crude extract containing plant aminoacyl-tRNA synthetases was applied to the column (Fig. 1). The three synthetases PheRS, MetRS and ArgRS were well separated. The assay for the elongation factor[11] was also carried out. For oligomeric AARS such as PheRS and MetRS from lupin seeds[18] or wheat germ[24,25] two peaks of activity are found, but with lupin ArgRS one major and one minor peak are present. This result could suggest that ArgRS appears here mainly as a monomer together with a small amount of dimer[25]. Very promising results were obtained in the purifica-

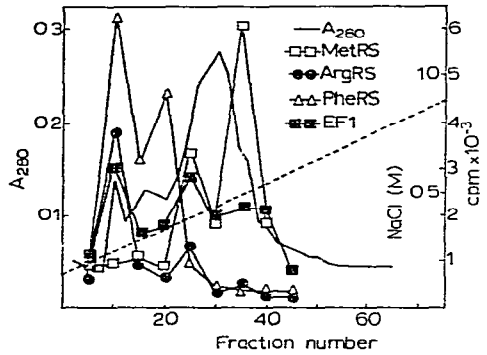

Fig. 1. Heparin–Sepharose CL 6B column (30 × 0.9 cm) chromatography of a crude protein extract after passage over DEAE-cellulose. The column was equilibrated with buffer D and eluted with a KCl gradient (25–500 m*M*) in buffer D. Total gradient volume was 220 ml. Fractions of 3 ml were collected at a flow-rate of 7.5 ml/h.

tion of PheRS and ArgRS from lupin seeds. The following scheme for the preparation of the pure enzyme was applied: Sephadex G-150, DEAE-Sephadex A-50 (Fig. 2) and heparin–Sepharose (Fig. 3). The final preparation showed a single band upon gel electrophoresis and the specific activity of ArgRS was 450 units. Similar results were obtained for PheRS from yellow lupin seeds. This is the first demonstration of the high performance of the heparin–Sepharose column in the purification of aminoacyl-tRNA synthetases.

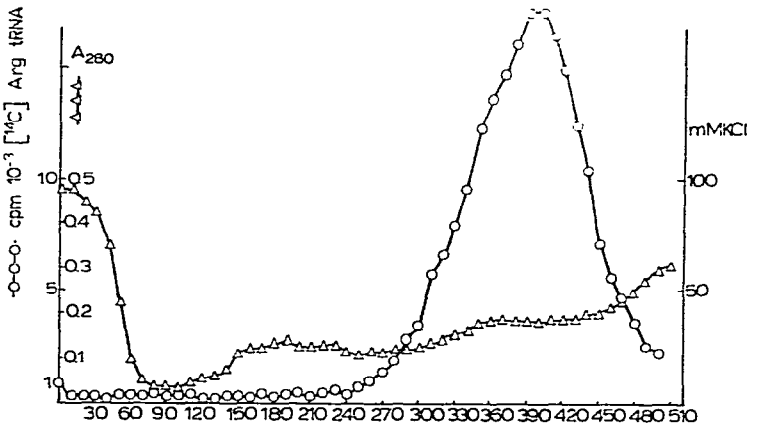

Fig. 2. DEAE-Sephadex A-50 column (70 × 2.3 cm) chromatography of arginyl-tRNA synthetase using the relevant fractions from a Sephadex G-150 column. The column was equilibrated with buffer B and eluted with a 1 l gradient of buffers B and C. Fractions of 2.5 ml were collected at a flow-rate of 10 ml/h.

It is not clear why pure AARS from plants[19] showed lower specific activity before the heparin-Sepharose chromatography. One can only speculate that heparin binds some compounds which block the activity of the AARS. With the available information on the chemical nature of the heparin molecule[21] and its inhibitory effects

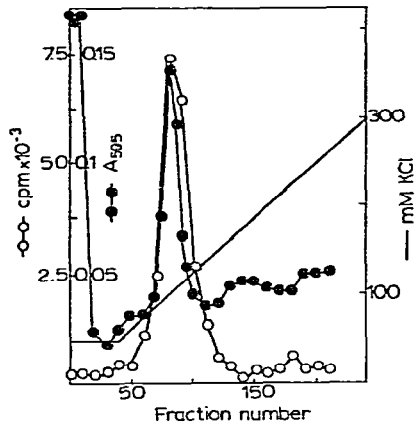

Fig. 3. Heparin–Sepharose CL 6B chromatography of arginyl-tRNA synthetase. The column (as in Fig. 1) was equilibrated with buffer D and eluted with a KCl gradient (50–300 m*M*) in buffer D. Total gradient volume was 400 ml. Fractions of 2.5 ml were collected ar a flow-rate of 7.5 ,ml/h.

on several other enzymes[26,27] we can proceed to investigate the interaction of heparin with aminoacyl-tRNA synthetases. This was accomplished by studying the effect of heparin on the charging of $tRNA^{Phe}$ with PheRS. Previous results[26] have suggested that heparin competes with nucleic acids. Our data are presented in Figs. 4 and 5. The Lineweaver–Burk plot (Fig. 4) showed a non-competitive mode of inhibition by

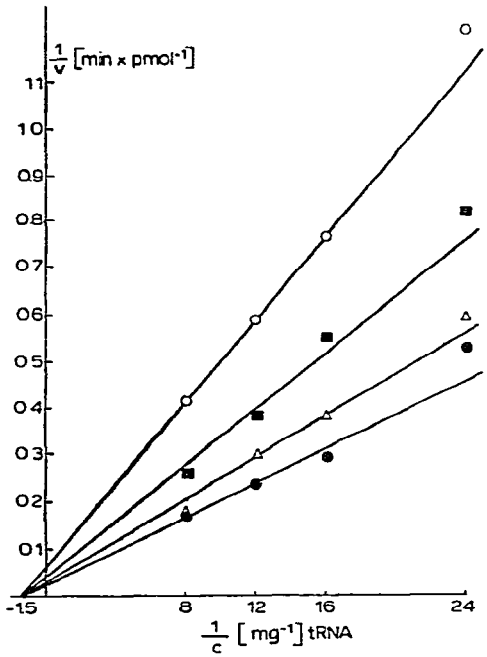

Fig. 4. Lineweaver–Burk plot of $tRNA^{Phe}$ charging with phenylalanine in the presence of heparin: ●—●, 0 mg/ml; △—△, 0.83 mg/ml; ■—■, 3.3 mg/ml; ○—○, 6.67 mg/ml.

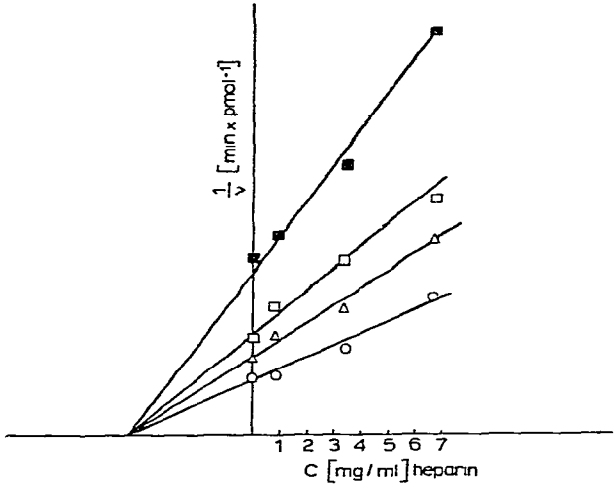

Fig. 5. Dixon plot of inhibition of aminoacylation of $tRNA^{Phe}$ in the presence of heparin. Concentration of tRNA: ○, 0.125 mg; △, 0.083 mg; □, 0.063 mg; ■, 0.042 mg per 0.15 ml.

heparin with respect to $tRNA^{Phe}$, indicating that the inhibitor is bound to the enzyme but not to a tRNA binding site. The same effect is seen from the Dixon plot[28]. The inhibitory effect of heparin on aminoacylation of tRNA is much weaker than, for example, its effect on the binding of aminoacyl-tRNA to ribosomes[26]. However, no effect on the binding of GTP to EF 1 or on the ability of the factor to form a ternary complex with GTP and AA-tRNA has been observed[26]. This suggests that heparin interferes with the ribosome attachment site in the ternary complex[26,27].

Finally, we conclude that heparin–Sepharose column chromatography offers a new possibility for the purification of aminoacyl-tRNA synthetases from higher organisms.

ACKNOWLEDGEMENTS

We thank Professor Maciej Wiewiórowski for his support, comments and criticisms and Mrs. Iwona Gawrońska for skillful technical assistance. Thanks are also due to Pharmacia Fine Chemicals for a gift of heparin–Sepharose CL 6B. This work was supported by the Polish Academy of Sciences within project 09.7.1.2.8.

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