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USE OF ω-AMINOHEXYL-SEPHAROSE IN THE FRACTIONATION OF *ESCHERICHIA COLI* B AMINOACYL-tRNA SYNTHETASES

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SUMMARY

The usefulness of aminohexyl-Sepharose in purification of *E. coli* B aminoacyl-tRNA synthetases is presented.

The purification factors for 14 synthetases lie in the range 3- to 94-fold and the recoveries of the enzymatic activity were 30–80%, depending on the enzyme.

INTRODUCTION

Classical procedures for the separation of proteins are usually based on differences in their solubility, charge, size and shape. Recently it was shown[1–4] that proteins can also be separated on the basis of their hydrophobicity, *i.e.*, the availability of their hydrophobic pockets. Thus, alkyl- and aminoalkyl-Sepharoses were recognized as useful adsorbents in protein separation and several enzymes were purified on these adsorbents[1–3,5–11]. One of these adsorbents, aminohexyl-Sepharose, has also been used for fractionation of transfer RNA[12]. In our hands, aminohexyl-Sepharose was superior to other adsorbents in the purification of plant aminoacyl-tRNA synthetases[3,11] and tRNA methyltransferases[9].

As *E. coli* is widely used as a source of many enzymes and since the purification of enzymes is often difficult, we felt that it was worthwhile to try to apply hydrophobic chromatography of aminohexyl-Sepharose for the purification of *E. coli* B aminoacyl-tRNAsynthetases. The resolution of 14 synthetases on aminohexyl-Sepharose columns is presented; purification factors for these enzymes were within the range 3–90-fold.

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EXPERIMENTAL

Materials

E. coli B was purchased from the Grain Processing Co., Muscatine, Iowa, U.S.A. and was stored at −20° until used. Sepharose 4B was purchased from Pharmacia (Uppsala, Sweden), CNBr from Pierce (Rockford, Ill., U.S.A.), 1,6-hexanediamine from Aldrich (Milwaukee, Wisc., U.S.A.), glass beads, streptomycin sulphate, ARP and mercaptoethanol from Sigma (St. Louis, Mo., U.S.A.) and *E. coli* B tRNA from Schwarz Bioresearch (Orangeburg, N.Y., U.S.A.). Uniformly labeled [^{14}C]-amino acids were purchased from the Radiochemical Centre (Amersham, Great Britain), and [^{3}H]amino acids from New England Nuclear (Boston, Mass., U.S.A.). Other reagents were of analytical-reagent grade.

Methods

Aminohexyl-Sepharose was prepared according to Cuatrecasas[13]; 100 mg of BrCN was used per millilitre of the gel. Protein was determined by the methods of Warburg and Christian[14] and Lowry *et al.*[15].

Assays of aminoacyl-tRNA synthetases were carried out in a reaction medium composed of 0.1 *M* tris–HCl (pH 7.8), 10 m*M* $MgCl_2$, 1 m*M* ATP, 1 m*M* mercaptoethanol, 4 mg/ml tRNA, 10^{-5}–$2.8 \cdot 10^{-4}$ *M* [^{14}C]- or [^{3}H]-amino acid and enzyme. After appropriate time intervals at 37°, radioactive aminoacyl-tRNA in 30- or 50-μl aliquots was determined by the filter-paper disc technique of Mans and Novelli[16]. One unit of enzyme activity is determined in the aminoacylation of 1 nmole of tRNA in 1 min at 36°.

The 40–60% ammonium sulphate saturation fraction was prepared from *E. coli* B as described by Lövgren *et al.*[17] and was dialysed against 60 m*M* potassium phosphate (pH 6.8), 7 m*M* mercaptoethanol and 10% (v/v) glycerol before application on to the aminohexyl-Sepharose column.

RESULTS AND DISCUSSION

In our earlier studies[3] we showed that plant aminoacyl-tRNA synthetases are not adsorbed on aminoethyl- and aminobutyl-Sepharose. However, these enzymes are adsorbed on an aminohexyl-Sepharose column and can be eluted from the column in a potassium chloride gradient. Our results also indicated that the main forces responsible for binding of aminoacyl-tRNA synthetases to aminohexyl-Sepharose were hydrophobic.

The successful application of aminohexyl-Sepharose in the purification of plant aminoacyl-tRNA synthetases led us to search for other applications of this adsorbent. We found that it was useful in the purification of tRNA methyltransferases[9] and in the fractionation of lupin tRNA[12].

Another application of aminohexyl-Sepharose columns is illustrated in this paper. *E. coli* B aminoacyl-tRNA synthetases are adsorbed on aminohexyl-Sepharose in 60 m*M* potassium phosphate buffer (pH 6.8) containing 10% of glycerol and 7 m*M* mercaptoethanol. The enzymes can be eluted in a linear potassium chloride gradient (Figs. 1 and 2). The elution profiles did not change when the size of the column was

increased 10-fold. The same order of elution of aminoacyl-tRNA synthetases was obtained on 50-ml (Fig. 1) and 500-ml (Fig. 2) columns.

The positions of some aminoacyl-tRNA synthetases were further confirmed by stepwise elution. For example, glutamyl-tRNA synthetase emerges in 0.1 *M* potassium chloride eluate, leucine enzyme in 0.2 *M* potassium chloride eluate and asparaginine enzyme in 0.3 *M* potassium chloride eluate. Thus, the order of elution of *E. coli* B aminoacyl-tRNA synthetases from the aminohexyl-Sepharose column was established as follows: 1, glutamyl-; 2, isoleucyl-; 3, aspartyl-; 4, tyrosyl-; 5, arginyl-; 6, glutaminyl-; 7, histidyl-; 8, valyl-, alanyl- and methionyl-; 9, phenylalanyl-; 10, leucyl-; 11, lysyl-; and 12, asparaginyl-tRNA synthetase. Yellow lupin aminoacyl-tRNA synthetases[3] are eluted under the same conditions in different positions on the gradient and the order of elution is 1, valyl- and lysyl-; 2, leucyl-; 3, tryptophanyl-; and 4, isoleucyl- and phenylalanyl-tRNA synthetase. Only phenylalanine enzymes from both sources are eluted in about the same position (0.25 *M* potassium chloride).

Comparison of the elution positions with the sizes of the enzyme reveals that aminoacyl-tRNA synthetases with more complex structures are eluted in higher ionic strength from the aminohexyl-Sepharose columns, with the exception of valyl-, leucyl- and isoleucyl-tRNA synthetases. It is conceivable that the larger the enzyme molecule the higher is the frequency of occurrence of hydrophobic pockets[3]. Thus we

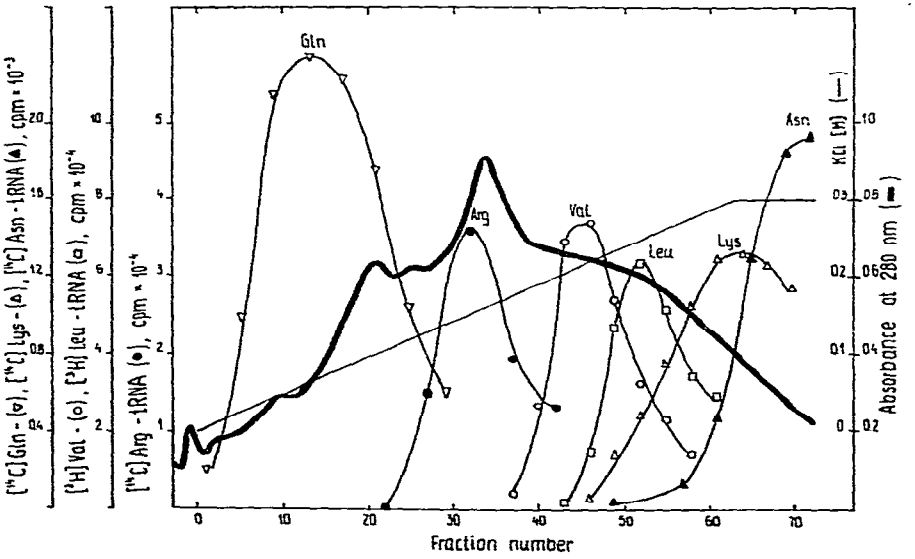

Fig. 1. Chromatography of *E. coli* B aminoacyl-tRNA synthetases on aminohexyl-Sepharose column. The column (1.5 × 25 cm) was equilibrated with 60 m*M* potassium phosphate (pH 6.8), 7 m*M* mercaptoethanol and 10% glycerol. Protein (15 ml; 900 mg) was applied on to the column. After washing with 100 ml of the buffer, the enzymes were eluted with a 0–0.3 *M* linear potassium chloride gradient in the buffer (total volume 512 ml) followed by 0.3 *M* potassium chloride. The flow-rate was 32 ml/h and 8-ml fractions were collected. The absorbance at 280 nm (thick curve) and the synthetase activities in 10-μl aliquots of fractions were determined: glutamyl- (▽), arginyl- (●), valyl- (○), leucyl- (□), lysyl- (△) and asparaginyl-tRNA synthetase (▲). Fractions 35–50, 45–55, 60–70 and 63–74 contain isoleucyl-, tyrosyl-, methionyl- and phenylalanyl-tRNA synthetase, respectively.

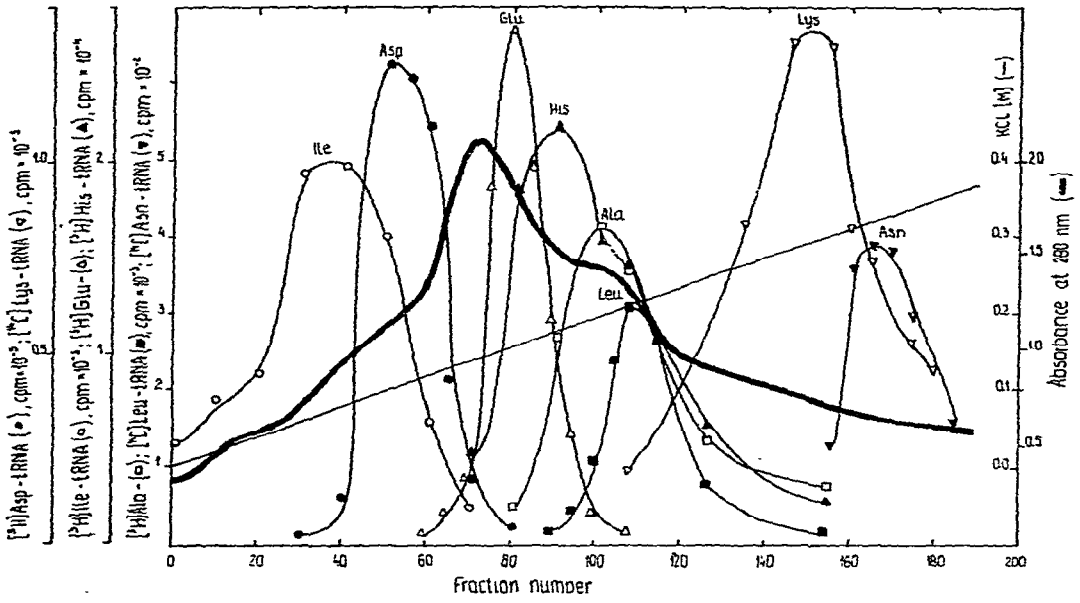

Fig. 2. Chromatography of *E. coli* B aminoacyl-tRNA synthetases on aminohexyl-Sepharose column. The column (5 × 25 cm) was equilibrated with 60 m*M* potassium phosphate (pH 6.8), 7 m*M* mercaptoethanol and 10% glycerol. Protein (190 ml; 13.3 g) was applied on to the column and the column was washed with 2900 ml of the buffer. The enzymes were eluted in a 0–0.4 *M* linear potassium chloride gradient in the buffer (total volume 5120 ml). The flow-rate was 320 ml/h and 25-ml fractions were collected. The absorbance at 280 nm (thick curve) and the synthetase activities in 10-μl aliquots of appropriate fractions were determined: isoleucyl- (○), aspartyl- (●), glutaminyl- (△), histidyl- (▲), alanyl- (□), leucyl- (■), lysyl- (▽) and asparaginyl-tRNA synthetase (▼).

could expect, at least with this class of enzymes, that an enzyme with a molecular weight above 150,000 daltons would be eluted from a hydrophobic column later than an enzyme with a molecular weight below 100,000 daltons. This was the case for yellow lupin enzymes[3] and also seems to be true for *E. coli* B enzymes: the aminoacyl-tRNA synthetases with molecular weights below 100,000 daltons are eluted below 0.2 *M* potassium chloride, whereas those with molecular weights above 150,000 daltons are eluted above 0.2 *M* potassium chloride (see Figs. 1 and 2; the molecular weights of *E. coli* aminoacyl-tRNA synthetases are listed in reference 18).

It was shown by Hofstee[19] that the binding behaviour of several proteins on DEAE-agarose is similar to that on certain-*n*-aminoalkyl-Agaroses. Indeed, aminohexyl-Sepharose resembles in some respects DEAE-cellulose (the order of elution of isoleucyl-, arginyl-, valyl- and leucyl-tRNA synthetases is the same for both adsorbents), but not in others (lysyl-tRNA synthetase is eluted after leucyl-tRNA synthetase from aminohexyl-Sepharose but before it on DEAE-cellulose). It is reasonable to propose that different features of proteins are engaged in binding to DEAE-cellulose (mainly ionic charges) and aminohexyl-Sepharose (mainly hydrophobic pockets).

The purification factors for the 14 aminoacyl-tRNA synthetases lie in the range 3–94-fold, and for 10 enzymes the purification factor is at least 10-fold (Table I). The recoveries of the enzymatic activity were 30–80%, depending on the enzyme. These results clearly illustrate the usefulness of aminohexyl-Sepharose in the purifica-

TABLE I

PURIFICATION OF AMINOACYL-tRNA SYNTHETASES FROM *E. COLI* B ON AMINOHEXYL-SEPHAROSE COLUMNS

*Aminoacyl-tRNA synthetase for**	*Specific activity of aminoacyl-tRNA synthetases (units/mg)*		*Purification factor*
	40–60% $(NH_4)_2SO_4$ satn.	*Aminohexyl-Sepharose*	
Gln	1.3	26	20
Ile	0.16	15	93.8
Asp	1.0	28	28
Tyr	1.2	7	5.8
Arg	3.4	18	5.3
Glu	0.13	2	15.4
His	0.2	2	10
Ala	0.7	2	2.9
Met	2.1	11	5.2
Val	4.2	53	12.6
Phe	1.6	16	10
Leu	2.6	55	21.2
Lys	5.2	130	25
Asn	1.2	15	12.5

* Aminoacyl-tRNA synthetases are listed in order of elution.

tion of *E. coli* B aminoacyl-tRNA synthetases. Combined with classical procedures for the separation of proteins, hydrophobic chromatography on aminohexyl-Sepharose could considerably improve the purification of aminoacyl-tRNA synthetases. The recovery of pure arginyl-tRNA synthetase from *E. coli* B was increased 4–5-fold by the introduction of aminohexyl-Sepharose column chromatography into the original purification procedure[20].

The operation of aminohexyl-Sepharose column is very convenient, and it can be regenerated simply by washing with 1 *M* sodium chloride solution. The flow-rates are about one column volume per hour.

In conclusion, we consider that aminohexyl-Sepharose is useful for procedures of the purification of aminoacyl-tRNA synthetases from many organisms.

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