Journal of Chromatography, 103 (1975) 364–367
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CHROM. 7801

Note

ω-Aminohexyl-Sepharose 4B, a new support for tRNA fractionation

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(Received July 10th, 1974)

Aminoalkyl-Sepharoses are often used in the preparation of specific sorbents for affinity chromatography[1,2]. The aminoalkyl residues serve to bind an appropriate ligand and to separate it away from the Sepharose matrix, thus facilitating the interactions between it and chromatographed molecules. It has been found, however, that aminoalkyl-Sepharoses and also alkyl-Sepharoses are able to bind protein macromolecules. Several enzymes have been purified on these adsorbents[3–8].

The forces responsible for the binding properties of aminoalkyl-Sepharoses and alkyl-Sepharoses are mainly hydrophobic. In this paper, we show that aminoalkyl-Sepharoses also bind tRNA, and that such binding is dependent on the length of the aminoalkyl residue. These properties of aminoalkyl-Sepharoses allowed us to fractionate yellow lupin tRNA on ω-aminohexyl-Sepharose 4B.

EXPERIMENTAL

All materials were as specified earlier[7]. Unfractionated tRNA from yellow lupin *(Lupinus luteus)* seeds was obtained by the method of Vanderhoef *et al.*[9]. Aminoacyl-tRNA synthetases from yellow lupin seeds (the fraction of 50–70% ammonium sulphate saturation was used as a source of tyrosine and serine synthetases and the other synthetases were obtained from the fraction of 35–50% ammonium sulphate saturation) were obtained by the method of Jakubowski and Pawełkiewicz[7].

The assay mixture for tRNA species contained, per 65 μl: 50 μl of column fraction (the fraction was dialyzed overnight against three changes of buffer containing 10 m*M* magnesium chloride and 20 m*M* Tris–hydrochloric acid, pH 7.5, then heated for 5 min at 60° in the same buffer before assaying for amino acid acceptance), 10 m*M* magnesium chloride, 1 m*M* ATP, 100 m*M* Tris–hydrochloric acid, pH 8.0, 1–2 nmoles ^{14}C- or ^{3}H-labelled amino acid and 1 μl of partially purified aminoacyl-tRNA synthetase.

Samples were incubated for 15 min at 37° and then 50-μl aliquots were withdrawn and pipetted on to Whatman 3MM filter-paper discs 2.2 cm in diameter. The discs were washed three times with 5% trichloroacetic acid, once in ethanol–diethyl ether (1:1) and finally in diethyl ether, then air dried. They were counted in a Packard Tri-Carb scintillation counter with an efficiency of 55% for ^{14}C and 5% for ^{3}H. An LKB Ultra-Rac, Uvicord II and recorder were used for fraction collection and recording of absorbance at 260 nm.

Chromatographic methods

The carrier, ω-aminohexyl-Sepharose 4B, was washed in a column (100 × 0.7 cm) with 2 *M* sodium chloride solution until the absorbance of the eluate at 260 nm was less than 0.01, and then equilibrated with starting buffer (0.4 *M* sodium chloride, 10 m*M* sodium acetate, pH 4.5).

Subsequently, 1000 A_{260} units of unfractionated tRNA from yellow lupin seeds were loaded on to the column. The tRNA was eluted in a linear gradient of sodium chloride (0.4–1.1 *M*, 500 ml) followed by a solution of sodium chloride (1.2 *M*, 200 ml) in buffer containing 10 m*M* sodium acetate, pH 4.5. Next, 1000 A_{260} units of unfractionated tRNA were chromatographed on the same column in a linear gradient of sodium chloride (0.25–0.58 *M*, 520 ml) in 10 m*M* sodium acetate, 7 *M* urea adjusted to pH 3.0 with hydrochloric acid. The columns were run at room temperature at flow-rates of about 30 and 15 ml/h, respectively. Fractions of 2.9 and 2.6 ml, respectively, were collected. The recoveries were nearly quantitative (95%) in terms of both absorbance and aminoacyl acceptors activities.

RESULTS AND DISCUSSION

As it had been shown that binding of proteins on aminoalkyl-Sepharoses is dependent on hydrocarbon chain length[7], we determined the sorption properties of aminoethyl-, aminobutyl-, and aminohexyl-Sepharose 4B for lupin tRNA. The results are summarized in Table I.

TABLE I

BINDING OF tRNA TO THE AMINOALKYL DERIVATIVES OF SEPHAROSE 4B

Three columns (1.0 × 10 cm) of aminoethyl-, aminobutyl- and aminohexyl-Sepharose were equilibrated with 10 m*M* sodium acetate, pH 4.5 and 0.1 *M* sodium chloride. Then 100 A_{260} units (1 ml) of unfractionated tRNA were applied on to each column and eluted with 0.1–1.0 *M* sodium chloride gradients (100 ml) in 10 m*M* sodium acetate buffer, pH 4.5. The strength of tRNA binding is expressed in terms of the sodium chloride concentration required for elution of the bulk of tRNA from the appropriate column.

Carrier	*NaCl concentration (M) required for elution of the bulk of tRNA*
Sepharose 4B–$NH(CH_2)_2NH_2$	0.35
Sepharose 4B–$NH(CH_2)_4NH_2$	0.45
Sepharose 4B–$NH(CH_2)_6NH_2$	0.70

Aminohexyl-Sepharose 4B binds the tRNA more strongly than do the other derivatives, aminoethyl-Sepharose 4B being the weakest adsorbent.

It was interesting to determine whether all tRNA species bind to aminoalkyl-Sepharose with the same strength. As the ionic strength required for elution of the bulk of tRNA was highest for aminohexyl-Sepharose 4B, we hoped that any differences in the strength of binding for tRNA species (or isoacceptors) would be much more easily detectable on this sorbent.

Fig. 1 shows the fractionation of lupin tRNA on ω-aminohexyl-Sepharose 4B. It is evident that the adsorbent has sufficient resolving power for fractionation of

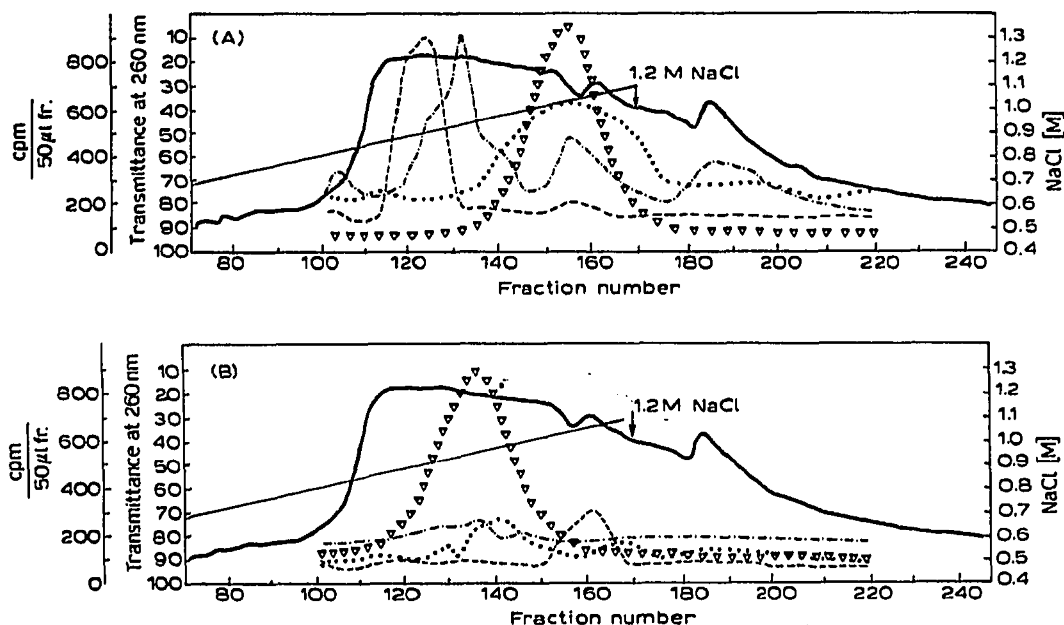

Fig. 1. Fractionation of tRNA from yellow lupin seeds on an ω-aminohexyl-Sepharose 4B column in a gradient of NaCl, pH 4.5 (0.4–1.1 *M*, 500 ml) followed by a solution of NaCl (1.2 *M*, 200 ml). Incorporation of the following aminoacids into tRNA was measured: (A) — — — [^{3}H]Val; ······ [^{14}C]Tyr; —·—·— [^{3}H]Ile; ▽▽▽ [^{3}H]Phe. (B) ▽▽▽ [^{14}C]Leu; — — — [^{14}C]Lys; ······ [^{14}C]Ser; —·—·— [^{14}C]Trp.

$tRNA^{Val}$ from $tRNA^{Phe}$ and $tRNA^{Lys}$, for fractionation of $tRNA^{Leu}$ from $tRNA^{Lys}$, and fractionation of $tRNA^{Ile}$ isoacceptors. The resolving power of ω-aminohexyl-Sepharose 4B may be improved by changing the elution conditions, *i.e.*, decreasing the pH and adding urea. As pointed out by Fitler *et al.*[10], the presence of urea interferes with hydrophobic interactions and decreasing the pH below the p*K* value of amino groups of adenylic and cytydylic acid residues changes the negative charge of the tRNA molecules, thus altering their tertiary structure.

As expected, in chromatography under acidic conditions in the presence of urea, the order of elution of the tRNAs is different from that under standard conditions (Fig. 2). These altered conditions resulted in the fractionation of $tRNA^{Val}$, $tRNA^{Ser}$, $tRNA^{Phe}$ into isoacceptors and the separation of $tRNA^{Trp}$ from the other tRNAs investigated.

The fractionation of tRNA on ω-aminohexyl-Sepharose 4B in 7 *M* urea and a sodium chloride gradient seemed to be critically dependent on the ratio of the column length to column diameter. The resolution to tRNA species was considerably impaired by increasing only the diameter of the column without a proportional increase in its length. The method gives reproducible results.

The profiles of tRNA resolved on ω-aminohexyl-Sepharose 4B columns are comparable to those on BD-cellulose[10,11] and RPC-5[12]. However, the peaks obtained with RPC-5 were much sharper than with the other two.

As the proteins adsorbed on ω-aminohexyl-Sepharose 4B are eluted at sodium

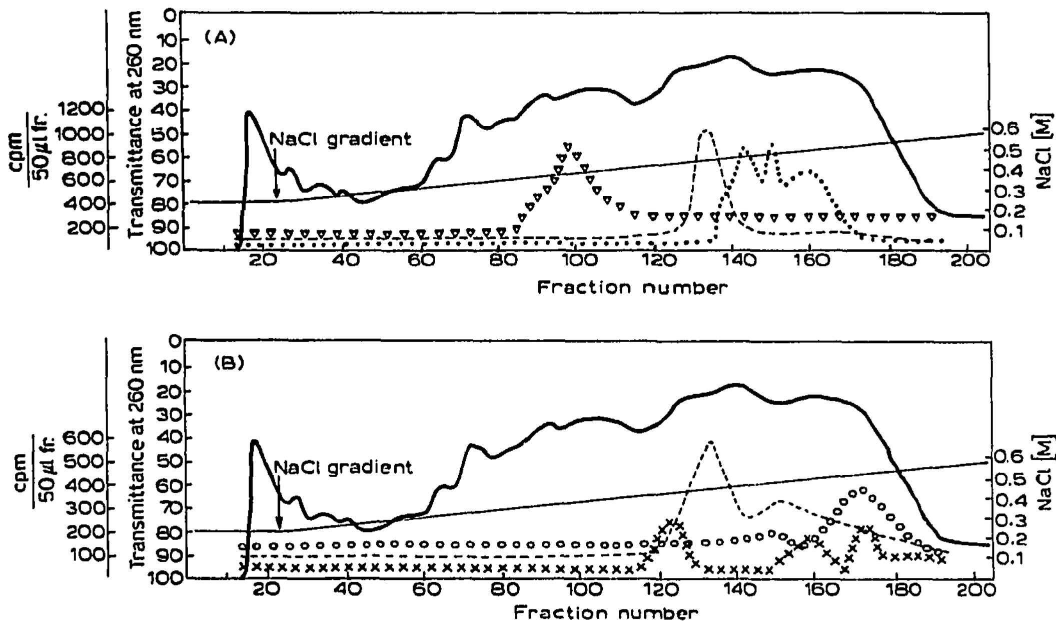

Fig. 2. Fractionation of tRNA from yellow lupin seeds on an ω-aminohexyl-Sepharose 4B column in 7 *M* urea, pH 3.0. Incorporation of the following aminoacids into tRNA was measured: (A) ▽▽▽ [^{14}C]Try; — — — [^{14}C]Tyr; ······ [^{3}H]Val. (B) — — — [^{3}H]Phe; ○○○ [^{14}C]Leu; × × × [^{14}C]Ser.

chloride concentrations below 0.4 *M* at pH 7.0, it is possible to use the adsorbent for purification (deproteinization) of unfractionated tRNA. In this respect, ω-aminohexyl-Sepharose 4B behaves like DEAE-cellulose but has a faster flow-rate. ω-Aminohexyl-Sepharose 4B seems to be a universal adsorbent for the purification of proteins (enzymes) and tRNAs.

ACKNOWLEDGEMENTS

The authors are greatly indebted to Prof. Dr. J. Pawełkiewicz for stimulating discussions and for revising the manuscript. This work was supported by the Polish Academy of Sciences, Project 09.3.1.

REFERENCES

1 P. Cuatrecasas, *J. Biol. Chem.*, 245 (1970) 3059.
2 M. Wilchek, *FEBS Lett.*, 33 (1973) 70.
3 Z. Er-el, Y. Zaidenzaig and S. Shaltiel, *Biochem. Biophys. Res. Commun.*, 49 (1972) 383.
4 S. Shaltiel and Z. Er-el, *Proc. Nat. Acad. Sci. U.S.*, 70 (1973) 778.
5 R. J. Yon, *Biochem. J.*, 137 (1974) 127.
6 Z. Er-el and S. Shaltiel, *FEBS Lett.*, 40 (1974) 142.
7 H. Jakubowski and J. Pawełkiewicz, *FEBS Lett.*, 34 (1973) 150.
8 B. H. J. Hofstee, *Biochem. Biophys. Res. Commun.*, 50 (1973) 751.
9 L. N. Vanderhoef, R. F. Bohannon and J. K. Key, *Phytochemistry*, 9 (1970) 2291.
10 F. Fitler, J. Kruppa and H. G. Zachau, *Biochim. Biophys. Acta*, 227 (1972) 513.
11 R. M. Kothari and M. W. Taylor, *J. Chromatogr.*, 73 (1972) 479.
12 R. M. Kothari and M. W. Taylor, *J. Chromatogr.*, 86 (1973) 289.