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## Note

# Temperature gradient chromatography of transfer RNA on hydroxyapatite

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Hydroxyapatite chromatography was first applied to fractionation of tRNA by Hartmann and Coy[1]. Since then, a number of methodological studies have appeared[2–8], which suggest that this method has a vast potential both for purification of individual species of tRNA and as an analytical tool for tRNA mixtures. Hydroxyapatite chromatography has been successfully applied to the purification of some tRNA species[7–13], although its use since then has been rather restricted. The reason for this is presumably two-fold. First, it is difficult to prepare batches of hydroxyapatite reproducibly. Second, the packing properties of hydroxyapatite columns changed rapidly with time, hence the same column gradually gave irreproducible results. The introduction of agar-entrapped hydroxyapatite has, however, made it possible to use repeatedly the column without change of properties.

The mechanism of interaction between tRNA and hydroxyapatite has not been conclusively elucidated. The work of Bernardi[14,15] and others indicates that the essential principle may be electrostatic interaction between calcium ions of the hydroxyapatite and phosphate groups of the tRNA. There is probably also an electrostatic repulsion between the phosphate groups of hydroxyapatite and the phosphodiester groups of the tRNA[6]. Therefore, the different elution properties of the individual tRNA species reflect the accessibility for electrostatic interaction of the phosphate groups in tRNA.

Any factor that affects the charge of the calcium or phosphate ions of the hydroxyapatite or the charge of steric accessibility of the phosphodiester groups of tRNA should influence the chromatographic pattern. It has been shown that a decrease in pH results in stronger binding of tRNA, possibly owing to decreased protonization of the phosphate groups of hydroxyapatite and hence a weakening of the phosphate–phosphodiester repulsion[6]. Also, the kind of positively charged counterions, which more or less neutralize the phosphodiester groups, strongly influences the elution pattern. Thus the binding of tRNA is much weaker in the presence of potassium phosphate buffer than in the presence of sodium phosphate buffer of the same pH and molarity[4,5].

Another parameter of interest is temperature. It has been demonstrated with several chromatographic systems utilizing ionic interaction that tRNA is more strongly adsorbed at higher temperatures[16–19]. This effect is ascribed to a loosening

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of the tertiary structure of tRNA with increasing temperature, rendering more phosphate groups accessible for ionic interaction. The same principle should be applicable to hydroxyapatite. In the present paper we show that a change in temperature in the range 5–25°C strongly influences the adsorption of tRNA on hydroxyapatite and that it is possible to obtain a highly selective elution pattern with a temperature gradient.

## EXPERIMENTAL

### *Materials*

L-[$^{14}$C]Amino acids were from NEN Chemicals and had the following specific activities, expressed as mCi/mmol: arginine, 300; aspartic acid, 167; glutamic acid, 200; histidine, 306; leucine, 291; lysine, 260; methionine, 55; phenylalanine, 383; proline, 213; serine, 128; tyrosine, 468; and valine, 260. HA-Ultrogel was from LKB, Stockholm, Sweden. *Escherichia coli* B was grown in medium E (20) with 0.5% glucose and 0.3% Nutrient broth (Difco), and tRNA and aminoacyl-tRNA ligase was prepared as previously described[21,22].

### *Aminoacylation of tRNA*

For the aminoacylation of tRNA in Fig. 1, the reaction mixture contained, in a final volume of 0.3 ml: 0.1 *M* Tris–HCl, pH 7.4; 2 m*M* ATP; 10 m*M* magnesium sulphate; 1 m*M* dithiothreitol; 1–4 $\mu M$ $^{14}$C-labelled amino acid; enzyme and 0.15 ml of the tRNA fraction. For the aminoacylation of tRNA in Fig. 2, the reaction mixture contained, in a final volume of 0.1 ml: 0.1 *M* Tris–HCl, pH 7.5 (for Arg, Lys, Ser) or 0.1 *M* N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (HEPES) buffer, pH 7.1 (for Phe), or 0.1 *M* HEPES buffer, pH 8.0 (for Leu), or 0.1 *M* sodium cacodylate, pH 7.1 (for Val); 2 m*M* ATP; 10 m*M* magnesium sulphate; 10 m*M* potassium chloride (for Lys, Ser, Val); 0.8–4 $\mu M$ $^{14}$C-labelled amino acid; enzyme and 50 $\mu$l of the tRNA fraction. After incubation at 37°C for 20 min, the samples were treated and measured as previously described[23].

## RESULTS AND DISCUSSION

Chromatography of tRNA from *E. coli* B was performed on a column of hydroxyapatite, using a combined phosphate concentration and decreasing temperature gradient. Fig. 1 shows the chromatogram obtained with hydroxyapatite prepared in our laboratory according to Tiselius *et al.*[24]. The distribution and the resolution of amino acid-specific tRNAs are very similar to that obtained by Pearson and Kelmers[4]. These authors also used hydroxyapatite prepared by the Tiselius method, but their chromatogram was developed by a phosphate gradient at constant room temperature. A comparison of these chromatograms shows that tRNA is readily eluted with lower salt concenterations at lower temperatures. For example, $tRNA^{Phe}$ is eluted with 0.17 *M* phosphate at 25°C and with 0.12 *M* at 4°C. The possibility of eluting with lower salt concentrations when using a temperature gradient may be of some advantage, because some aminoacyl-tRNA ligases are inhibited considerably by phosphate in higher concentrations.

There are some differences in the relative order of the tRNA species between

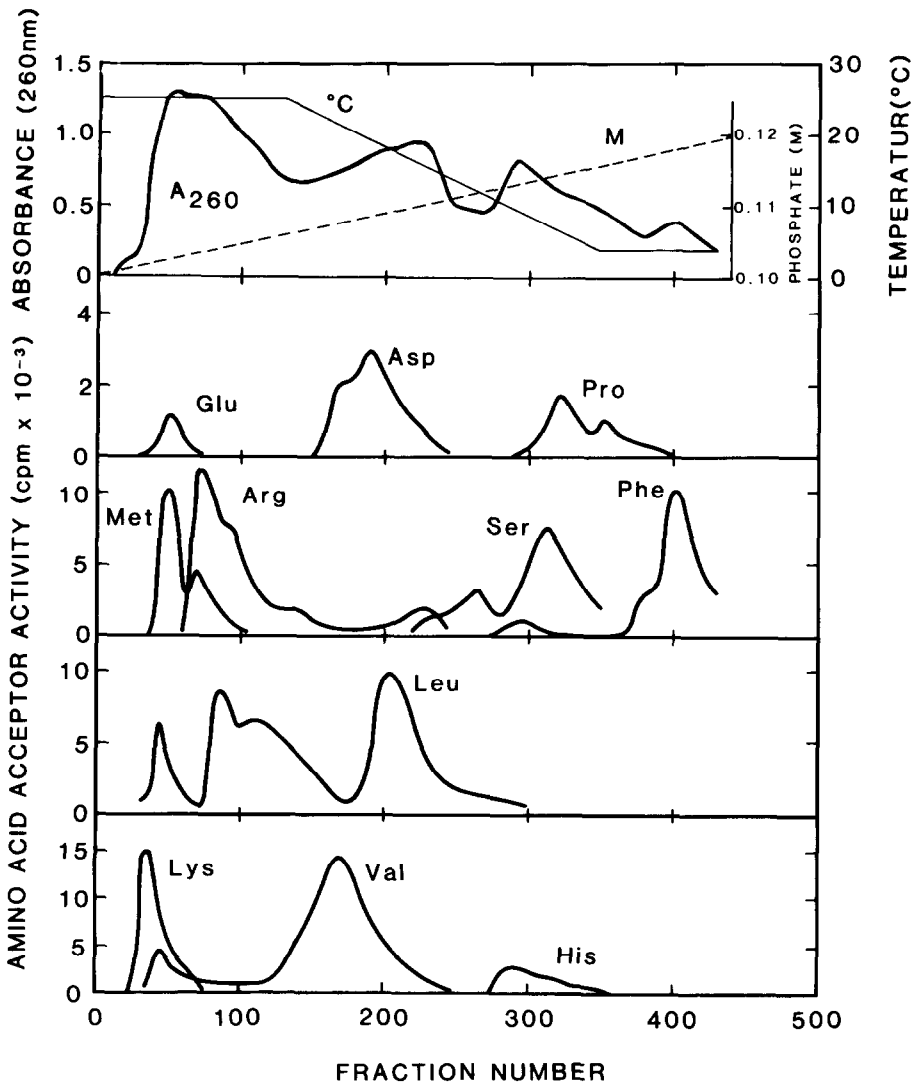


Fig. 1. Chromatography of *E. coli* tRNA on hydroxyapatite with a combined phosphate concentration and decreasing temperature gradient. The hydroxyapatite was prepared according to Tiselius *et al.*[24]; 200 mg of tRNA was applied to a 3.14 cm² × 86 cm column, pre-equilibrated at 25°C with 0.10 *M* sodium phosphate buffer, pH 6.8. The tRNA was eluted with a 3.5 + 3.5 l linear gradient of 0.10–0.12 *M* sodium phosphate buffer, pH 6.8. The flow-rate was 48 ml/h, and fractions of 16 ml were collected. Amino acid acceptor activity is expressed in cpm per 150 μl of sample except for arginine, where calculated values for cpm per 75 μl of sample are given.

our chromatogram in Fig. 1 and that of Pearson and Kelmers. We found no trace of $tRNA^{Pro}$ and $tRNA^{Ser}$ in the regions where they report proline 1 and serine 1, respectively. Our proline and serine acceptors are located in two peaks each in the later part of the chromatogram. The elution order between valine and aspartic acid acceptors is reversed.

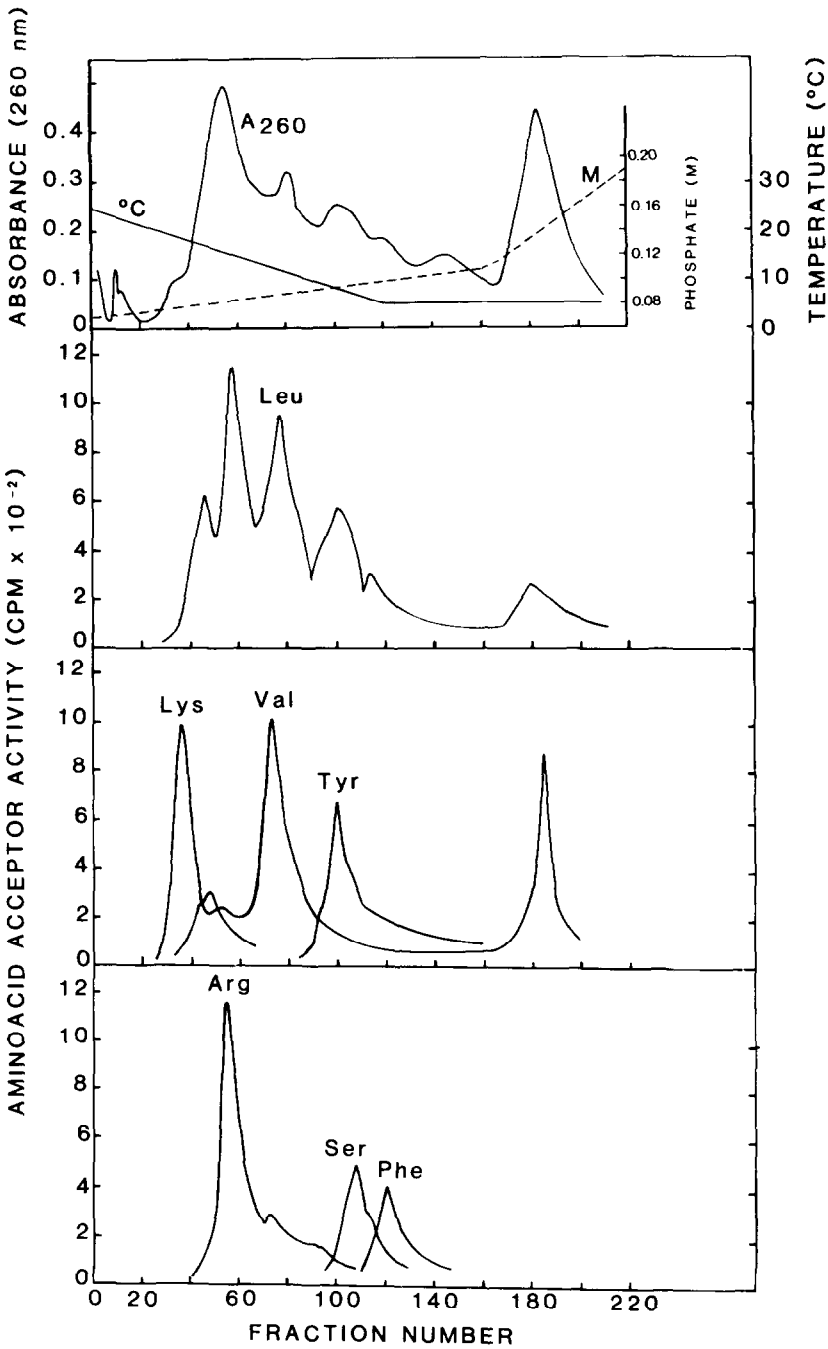


Fig. 2. Chromatography of *E. coli* tRNA on hydroxyapatite-agarose gel (HA-Ultrogel) with a combined phosphate concentration and decreasing temperature gradient; 8 mg of tRNA was applied to a 0.95 $cm^2$ × 53 cm column, pre-equilibrated at 25°C with 0.07 *M* sodium phosphate buffer, pH 6.8. The tRNA was eluted with 400 + 400 ml linear gradient of 0.07–0.11 *M* sodium phosphate buffer, pH 6.8. The flow-rate was 15 ml/h, and fractions of 5 ml were collected. After tube 156, tRNA was eluted further with 150 + 150 ml linear gradient of 0.11–0.20 *M* of the same buffer. Amino acid acceptor activity is expressed in cpm per 50 $\mu$l of sample.

Our chromatogram shows four peaks for $tRNA^{Leu}$, which correspond well to the first four leucine acceptors of Pearson and Kelmers. The region after tube 300 in our chromatogram, where the fifth leucine acceptor would be expected, was not assayed for leucine acceptance. These differences in elution order indicate that the effect of temperature on the elution from hydroxyapatite differs between tRNA species.

We have examined the agar-entrapped hydroxyapatite marketed under the trade name HA-Ultrogel as a medium for tRNA fractionation. Fig. 2 shows the distribution of some tRNAs using a combined phosphate concentration and a decreasing temperature gradient deviating somewhat from the conditions in Fig. 1. The temperature was lowered earlier, and a steeper salt gradient was applied late in the run to elute a sixth $tRNA^{Leu}$ and a fourth $tRNA^{Val}$ fraction. Again, there are some differences between the chromatograms of Fig. 1 and Fig. 2 with respect to the elution order. The major $tRNA^{Val}$ and $tRNA^{Ser}$ elute differently in relation to the $tRNA^{Leu}$ pattern.

The HA-Ultrogel column has been used about twenty times without detectable changes in reproducibility and stability. thus, with a reliable column material available, hydroxyapatite chromatography under conditions of controlled temperature seems to be still useful for fractionation of tRNA. Variations in the controlled combination of the two eluting factors, the phosphate concentration and the temperature, may render hydroxyapatite chromatography of tRNA highly adaptable to the specific needs of an analytical or a purification procedure.

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