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HYDROPHOBIC INTERACTION CHROMATOGRAPHY OF INCOMPLETELY METHYLATED TRANSFER RNA FROM *ESCHERICHIA COLI* ON OCTYL-SEPHAROSE

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SUMMARY

Phenylalanine-specific transfer RNA from methionine-starved relaxed *Escherichia coli* K12 separates into two components when chromatographed on Octyl-Sepharose. The difference in elution between the two tRNAs has been shown to depend on the methyl group in the highly modified 2-methylthio-N-6-isopentenyladenosine. The first eluted $tRNA^{Phe}$ lacks this methyl group, while the last eluted $tRNA^{Phe}$ is fully methylated. Other differences in the modification patterns have no effect on the elution from Octyl-Sepharose. The elution pattern of tyrosine- and serine-specific tRNAs, also normally containing ms^2i^6A, is similar.

INTRODUCTION

The biosynthesis of transfer RNA in microorganisms under exponential growth conditions results in a characteristic pattern of modified nucleosides, which is usually complete. This pattern may be disturbed if the cells are subjected to unbalanced growth. The presence of antibiotics, limitation of essential nutrients such as amino acids and iron, reduced aeration and extreme temperatures may thus lead to the appearance of tRNA species that are unmodified at specific sites in their structures[1–9]. The study and use of such incompletely modified tRNAs has contributed much to our present understanding of the properties and the biological rôle of the modified nucleosides in tRNA. Knowledge of the properties of the modified nucleosides in tRNA *in situ* facilitates for the development of separation methods and analytical techniques for the various incompletely modified tRNA species.

We have found[10] that phenylalanine-specific tRNA from methionine-starved relaxed *Escherichia coli* separates into two components when chromatographed on Octyl-Sepharose. The last of these peaks to be eluted represents normal $tRNA^{Phe}$, synthesized before the onset of methionine starvation, while the first peak eluted represents tRNA synthesized under methionine deficiency and is therefore unmethylated. The difference in elution should therefore be due to some hydrophobic character incorporated into tRNA during synthesis and maturation in the presence of meth-

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ionine. We report here that this character resides in the methylthio group of the complex modification N-6-(Δ^2-isopentenyl)-2-methylthioadenosine.

MATERIALS AND METHODS

Chemicals

L-^{14}C(U)-labelled amino acids of arginine (318 mCi/mmol), aspartic acid (167 mCi/mmol), phenylalanine (536 mCi/mmol), serine (151 mCi/mmol) and tyrosine (468 mCi/mmol) were obtained from New England Nuclear, L-[^{3}H(U)]phenylalanine (33 Ci/mmol), L-[^{14}C (U)]valine (265 mCi/mmol) and L-[^{3}H (U)]valine (33 Ci/mmol) from Amersham International. L-[^{3}H (U)]Valine was diluted in cold L-valine so as to obtain a specific activity of 656 mCi/mmol. Sepharose 6B, DEAE-Sepharose and Octyl-Sepharose CL-4B (Lot No. 13857) were from Pharmacia (Uppsala). Bacto-tryptone and Bacto-yeast extract were from Difco Laboratories. All the other chemicals used were of reagent grade.

Bacterial strains and growth conditions

Escherichia coli B and IB5[11] were grown in a rich medium as described[12] and harvested in the late logarithmic phase. *E. coli* K12, strain 58-161, RC^{rel}, met$^-$ (known as W6) was grown in minimal medium with a limiting amount of DL-methionine (10–15 mg/l) and harvested as described earlier[13]. *E. coli* K12, strain W3110 Lac Z U118 trp R trp X azi^R (called miaA mutant, previously known as trp X mutant) was kindly provided by Dr. Charles Yanofsky (Stanford University) and grown as described[14]. *E. coli* GRB 56, RC^{rel}, cys$^-$ was kindly provided by Professor Glenn Björk (Umeå, Sweden) and grown in minimal medium[12] containing limiting amounts of sulphate ($1.57 \cdot 10^{-5}$ *M*) and L-cysteine (10 mg/l). The cells were harvested after 3 h in the stationary phase.

Preparation of tRNA

Bulk tRNA was prepared from all bacterial strains by phenol extraction and ethanol precipitation[15]. After deacylation at 37°C for 1 h in 1 *M* Tris–HCl, pH 9.0, high-molecular-weight RNA was removed by chromatography on Sepharose 6B[16]. The tRNA was precipitated by adding two volumes of cold 95% ethanol, washed once with cold 70% ethanol, dried under vacuum and finally dissolved in distilled water and stored at −20°C.

Preparation of aminoacyl-tRNA ligases

A crude extract of aminoacyl-tRNA ligases was prepared from *E. coli* B according to Muench and Berg[17]. The preparation was stored at −70°C in portions of 0.1 ml.

Preparation of radioactive aminoacyl-tRNA

For phenylalanine incorporation, the reaction mixture contained, in a final volume of 0.5 ml, 0.1 *M* 4-(2-hydroxyethyl)-1-piperazine ethanesulphonic acid (HEPES) buffer, pH 7.1, 2 m*M* ATP, 10 m*M* magnesium sulphate, 1 m*M* dithiothreitol (DDT), 5 m*M* ammonium chloride, 1.9 μM [^{14}C]phenylalanine or 0.6 μM [^{3}H]phenylalanine, an appropriate amount of ligase and 3–40 A_{260} units of total

tRNA. For valine incorporation, the reaction mixture was 0.1 *M* sodium cacodylate, pH 7.1, 2 m*M* ATP, 10 m*M* magnesium acetate, 10 m*M* potassium chloride, 3.8 μM [^{14}C]valine or 10 μM [^{3}H]valine, ligase and 5–40 A_{260} units of total tRNA. After incubation at 37°C for 20 min, the reaction was terminated by adding 50 μl of 1 *M* sodium acetate buffer, pH 5.0, and the mixture chromatographed on DEAE-Sepharose[18]. The aminoacyl-tRNA was co-precipitated with carrier tRNA (200–400 A_{260} units) by ethanol, dried and dissolved in 0.01 *M* sodium acetate buffer, pH 5.0, containing 2.0 *M* ammonium sulphate.

Aminoacylation of fractionated tRNA

The aminoacylation mixture contained, in a final volume of 0.1 ml, 0.1 *M* HEPES, pH 7.1 (for Asp, Phe, Tyr), or 0.1 *M* Tris–HCl, pH 7.5 (for Arg, Ser), 2 m*M* ATP, 10 m*M* magnesium sulphate, 1 m*M* DTT and 5 m*M* ammonium chloride (for Asp, Phe, Tyr), 10 m*M* potassium chloride (for Ser), 0.2–1.5 μM ^{14}C-labelled amino acids, 25 μl of column fractions and an appropriate amount of aminoacyl-tRNA ligases. The mixture was incubated for 20 min at 37°C. The samples were treated as described[19].

Chromatography of tRNA on Octyl-Sepharose CL-4B

Octyl-Sepharose CL-4B was packed into a column (33 cm × 1.8 cm) and equilibrated against 0.01 *M* sodium acetate buffer, pH 5.0, containing 2.0 *M* ammonium sulphate, at a flow-rate of 24 ml/h. The column was then loaded with 500 A_{260} units of uncharged tRNA (for postlabelling experiment) or a mixture of 200–400 A_{260} units of uncharged tRNA with 5–40 A_{260} units of radioactive aminoacyl-tRNA (for prelabelling and double-labelling experiments). Elution was performed at room temperature with a negative salt gradient. The mixing chamber contained 350 ml of 0.01 *M* sodium acetate buffer, pH 5.0, containing 2.0 *M* ammonium sulphate, and the reservoir contained 350 ml of 0.4 *M* ammonium sulphate in the same buffer. Fractions of 2.4 ml were collected. The absorbance at 260 nm was measured and the conductivity was determined (in a water-bath) at 25°C. For measurement of prelabelled tRNA, aliquots of 1 ml were withdrawn and treated as described[19].

Permanganate oxidation of tRNA

tRNA was treated with potassium permanganate by the method of Kline *et al.*[20]. 100 A_{260} units of tRNA in 0.3 ml of water were treated for 5 min at 25°C with 0.1 ml of 0.1% potassium permanganate. The unreacted permanganate was destroyed by the addition of solid sodium bisulphite. The tRNA was precipitated (in potassium acetate and ethanol), washed and dried as described above, and dissolved in 0.2 ml of water.

RESULTS

Transfer RNA from methionine-starved *E. coli* K12 58-161 (W6) was chromatographed on Octyl-Sepharose under conditions where the individual tRNAs are eluted in order of increasing hydrophobicity. $tRNA^{Phe}$ was eluted into two, well separated peaks of about equal size (Fig. 1a). This was an indication that the hydrophobic Octyl-Sepharose could separate normal, fully methylated $tRNA^{Phe}$ and un-

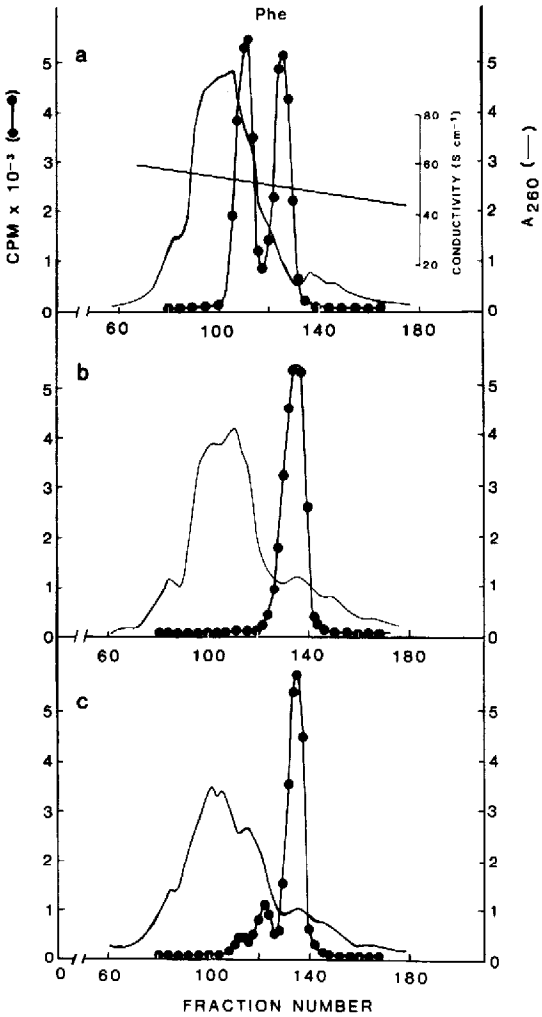

Fig. 1. Chromatography of total tRNA on Octyl-Sepharose. Fractions were analyzed for phenylalanyl-accepting activity after elution (postlabelling). ●—●, [^{14}C]phenylalanyl-tRNA. (a) Methionine-starved *E. coli* K12, strain 58-161, RC^{rel}, met$^-$ (W6); (b) *E. coli* B; (c) *E. coli* mutant IB5, trmA$^-$, deficient in m^{5}U.

methylated tRNA^{Phe}, which appear in about equal amounts after a few hours starvation for methionine in RC^{rel} mutants such as W6[21].

When normal, fully methylated tRNA from *E. coli* B was chromatographed on Octyl-Sepharose under the same conditions, only one peak for tRNA^{Phe} was obtained (Fig. 1b). This peak was eluted in an identical manner to the second tRNA^{Phe} peak from methionine-starved bacteria, both in relation to the UV pattern of total tRNA and the salt gradient. Thus we assume that the first tRNA^{Phe} peak eluted from methionine-starved bacteria represents unmethylated (or incompletely methylated) material.

The modified nucleosides in tRNA^{Phe} known to be directly affected by meth-

ionine starvation are 5-methyluridine (m^5U or T), 7-methylguanosine (m^7G), 3-(3-amino-3-carboxypropyl)uridine (X) and N-6-(Δ^2-isopentenyl)-2-methylthioadenosine (ms^2i^6A). The presence of any of these modifications in a tRNA might render the molecule more hydrophobic, either through their own hydrophobic nature, or in combination with other hydrophobic groups, or perhaps by inducing a conformational change to the molecule that will in turn expose other hydrophobic groups for interaction. The following experiments were designed to demonstrate which of these modifications participate in the chromatographic shift on Octyl-Sepharose as shown in Fig. 1a.

Effect of m^5U

Transfer RNA from the *E. coli* mutant IB5 is completely devoid of m^5U but has, as far as is known, the normal set of other modified nucleosides. When this tRNA was chromatographed on Octyl-Sepharose, only one peak of $tRNA^{Phe}$ appeared. It was eluted like normal *E. coli* B $tRNA^{Phe}$ (Fig. 1c). This shows that the methyl group of m^5U does not add to the hydrophobicity of $tRNA^{Phe}$.

Effect of m^7G

If the shift to early elution of $tRNA^{Phe}$ were a consequence of the absence of the methyl group of m^7G, then an elution shift might be expected to occur also for other tRNAs normally containing m^7G after methionine starvation of mutant W6. However, this is not the case for $tRNA^{Asp}$ and $tRNA^{Arg}$, which both contain m^7G. Fig. 2 shows that these tRNAs have the same elution pattern whether they contain

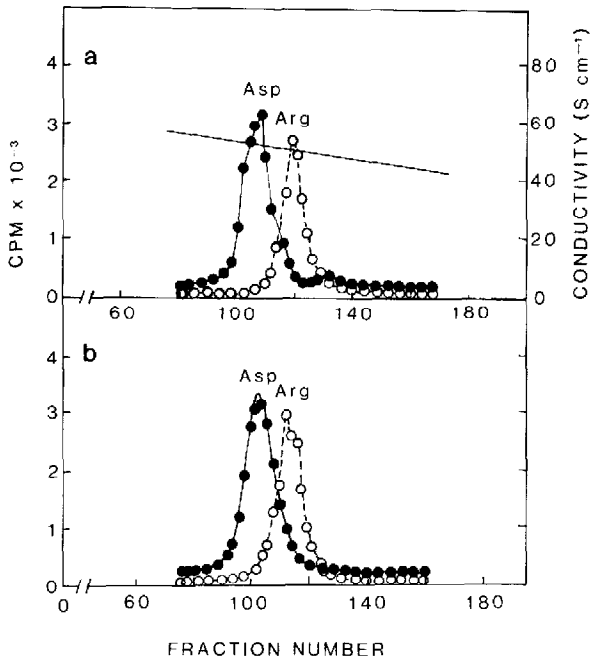

Fig. 2. The same chromatographic experiment as in Fig. 1. ●—●, [^{14}C]aspartyl-tRNA; ○---○, [^{14}C]arginyl-tRNA, both postlabelled. (a) tRNA from *E. coli* B; (b) tRNA from methionine-starved *E. coli* W6.

their normal modified nucleosides or lack the methionine-dependent nucleosides. We conclude that the methyl group of m^7G does not add to the hydrophobicity of $tRNA^{Asp}$ and $tRNA^{Arg}$. Probably, this conclusion is valid for $tRNA^{Phe}$ as well.

Effect of X

The modified nucleoside X derives its 3-amino-3-carboxypropyl group from methionine[22]. A tRNA synthesized during a period of methionine starvation in a RC^{rel} mutant of *E. coli* would therefore lack this modification. *E. coli* $tRNA^{Arg}$ normally contains the modification X but has no ms^2i^6A. Since this tRNA from methionine-starved *E. coli* W6 showed the same elution pattern as that of normal $tRNA^{Arg}$ (Fig. 2), we conclude that X has no influence on the hydrophobicity of tRNA.

Effect of ms^2i^6A

For the remaining methionine-dependent modification, ms^2i^6A, we have utilized the *E. coli* miaA mutant, which almost completely lacks both the methylthio group and the isopentenyl group in tRNA at site 37[14,23]. The effect of the strongly hydrophobic isopentenyl group will of course be in addition to that of any possible hydrophobic effect of the methylthio group. This is evident from Fig. 3a and b, where we compare Octyl-Sepharose chromatograms of tRNA from the miaA mutant and from methionine-starved mutant W6. The absence of both methylthio and isopentenyl groups has a much more pronounced effect on the hydrophobicity of the $tRNA^{Phe}$ molecule than the absence of the methyl group at site 37.

It should be noted that in Fig. 3 the tRNA was aminoacylated with [^{14}C]phenylalanine before chromatography (prelabelled), while in Figs. 1 and 2, tRNA was first chromatographed and then aminoacylated (postlabelled). Since phenylalanine is hydrophobic, Phe-tRNA should be eluted later than $tRNA^{Phe}$. A comparison between Figs. 1a and 3a verified this prediction. It can also be seen that the resolution between unmethylated and fully methylated $tRNA^{Phe}$ is almost the same whether the tRNA is aminoacylated or not.

From the elution pattern of miaA mutant $tRNA^{Phe}$ we can infer that the chromatographic shift may depend either on the absence of the isopentenyl group only, or on the absence of both the methylthio and the isopentenyl groups. The elution pattern of $tRNA^{Phe}$ from methionine-starved *E. coli* W6 seems to support the latter alternative. To discriminate further between these possibilities, we chromatographed Phe-tRNA from cysteine-starved *E. coli* GRB 56. This tRNA is a mixture of thiolated and non-thiolated species. The non-thiolated $tRNA^{Phe}$ must also be devoid of the methyl group at position 37. It is clear from Fig. 3c that $tRNA^{Phe}$ lacking the whole ms^2 group is eluted with the same pattern as $tRNA^{Phe}$ from methionine-starved bacteria (Fig. 3a). This result therefore supports a combined, cooperative hydrophobic effect in normal *E. coli* $tRNA^{Phe}$ of the methylthio and the isopentenyl groups.

Behaviour of permanganate-treated tRNA

Under mild conditions, permanganate is a specific reagent for cleavage of the isopentenyl group of tRNA[20], resulting in a much less hydrophobic residue. We have used this technique in combination with chromatography on Octyl-Sepharose to indicate the presence or absence of isopentenyl groups in a tRNA.

Unmethylated tRNA isolated by Octyl-Sepharose chromatography of meth-

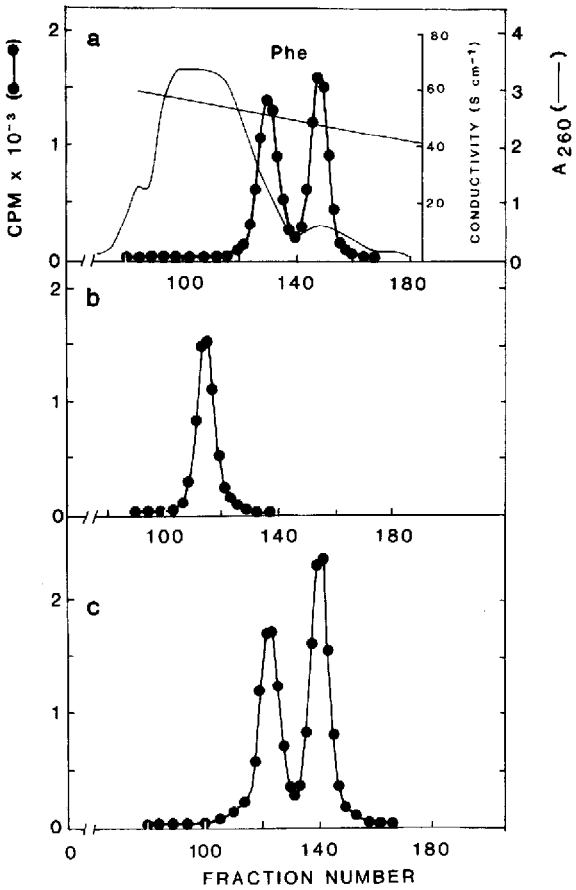

Fig. 3. Chromatography on Octyl-Sepharose of total tRNA, prelabelled with [^{14}C]phenylalanine. (a) Methionine-starved *E. coli* W6; (b) *E. coli* mutant miaA; (c) cysteine-starved *E. coli* GRB 56, RC^{rel}, cys$^-$.

ionine-starved *E. coli* W6 tRNA was treated with permanganate and subsequently aminoacylated with [^{3}H]phenylalanine. Untreated tRNA from the same batch was simultaneously aminoacylated with [^{14}C]phenylalanine. The two Phe-tRNAs were cochromatographed on Octyl-Sepharose. Fig. 4a shows that the oxidized tRNA^{Phe} was eluted earlier than untreated tRNA^{Phe}. This indicates that methionine starvation of *E. coli* W6 does not result in an isopentenyl deficiency. To confirm that the permanganate did not interfere with other hydrophobic centres in the tRNA molecule, we also chromatographed a mixture of permanganate-oxidized and untreated *E. coli* miaA tRNA, prelabelled with [^{3}H]- and [^{14}C]phenylalanine, respectively. These tRNAs were eluted identically as shown in Fig. 4b. The result confirms a report that tRNA^{Phe} from methionine-starved *E. coli* W6 contains the isopentenyl residue[24].

To establish that tRNA^{Phe} from cysteine-starved *E. coli* GRB 56 lacks the ms^2 group only and not the isopentenyl group, we also cochromatographed permanganate-treated and untreated Phe-tRNA, differently labelled, on Octyl-Sepharose (Fig. 4c). The result shows clearly that tRNA^{Phe} from cysteine-starved *E. coli* GRB 56 contains the isopentenyl residue, and this was confirmed by Agris *et al.*[25].

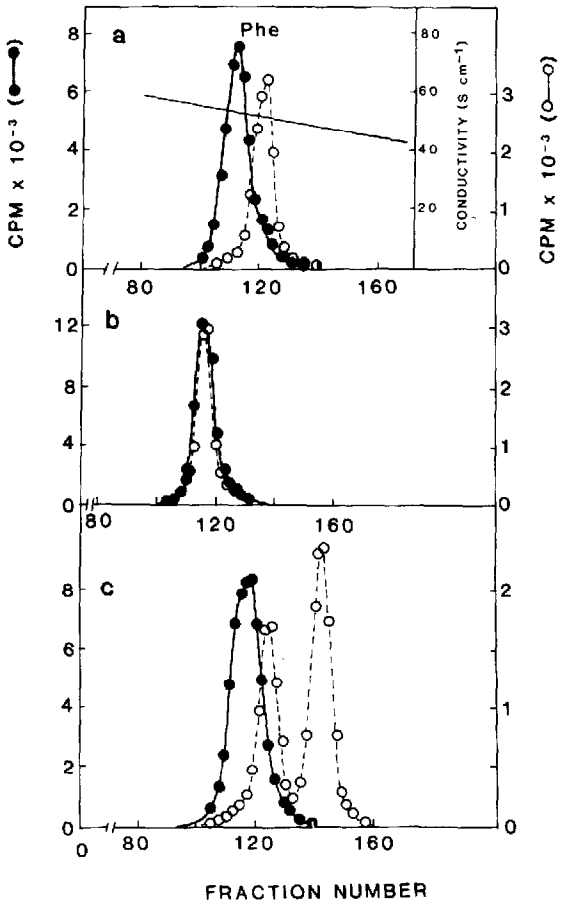

Fig. 4. Cochromatography on Octyl-Sepharose of prelabelled [^{3}H]phenylalanyl-tRNA, previously oxidized with permanganate (●—●), and prelabelled untreated [^{14}C]phenylalanyl-tRNA (○---○). (a) tRNA from methionine-starved *E. coli* W6; the material was from the left peak of Fig. 1a, (b) *E. coli* mutant miaA; (c) cysteine-starved *E. coli* GRB 56.

It may be noted that Phe-tRNAs resulting from methionine starvation and from cysteine starvation are eluted almost identically (*cf.*, Fig. 4a and c). There are two possible explanations for this. First, the methionine-starved $tRNA^{Phe}$ may contain the incomplete modification s^2i^6A and the cysteine-starved $tRNA^{Phe}$ may contain i^6A. If so, the presence of the thio group does not contribute to the hydrophobicity. Secondly, $tRNA^{Phe}$ from methionine starvation may be devoid of both the methyl and the thio groups and would then be identical with $tRNA^{Phe}$ from cysteine starvation at site 37. Our results do not permit a discrimination between these possibilities. However, Isham and Stulberg[24] reported that methionine starvation actually results in a reduction of ms^2i^6A to i^6A lacking both methyl and thio groups, while Agris *et al.*[25] found both i^6A and s^2i^6A.

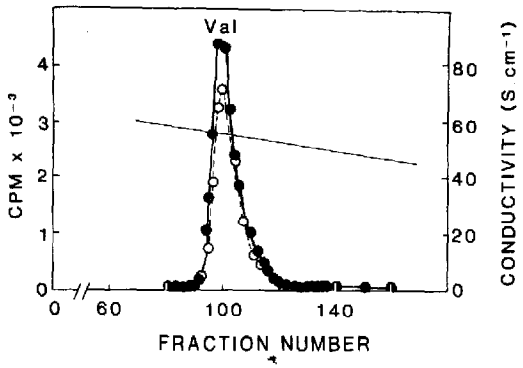

Fig. 5. Cochromatography on Octyl-Sepharose of prelabelled [^{3}H]valyl-tRNA from cysteine-starved *E. coli* GRB 56 (○—○), and [^{14}C]valyl-tRNA from unstarved *E. coli* GRB 56 (●—●).

Effect of s^4U

In order to exclude the possibility that 4-thiouridine present at site 8 in *E. coli* $tRNA^{Phe}$ might directly or indirectly influence the elution from Octyl-Sepharose, we chromatographed $tRNA^{Val}$ from both cysteine-starved and normal *E. coli* GRB 56. Fig. 5 shows that these tRNAs, prelabelled with valine, are eluted identically from

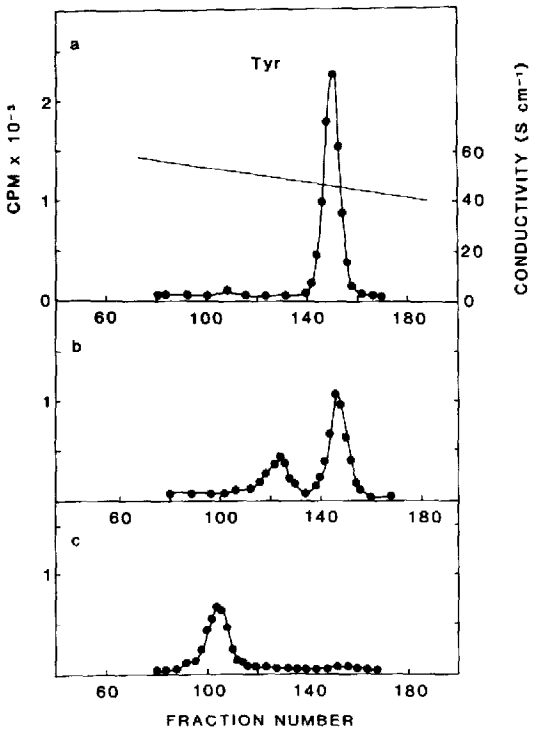

Fig. 6. Chromatography on Octyl-Sepharose of tRNA from (a) normal *E. coli*, (b) methionine-starved *E. coli* W6 and (c) *E. coli* miaA, respectively; the tRNA was postlabelled with [^{14}C]tyrosine.

Octyl-Sepharose. Since *E. coli* $tRNA^{Val}$ has no other sulphur-containing nucleoside besides s^4U_8, we conclude that this thio group does not contribute to the hydrophobic character of tRNA.

Behaviour of $tRNA^{Tyr}$ and $tRNA^{Ser}$

Besides $tRNA^{Phe}$, some *E. coli* isoacceptor tRNAs for tyrosine, serine, cysteine, tryptophan and leucine have been reported to contain ms^2i^6A at position 37[26]. To confirm our results with $tRNA^{Phe}$, we also studied $tRNA^{Tyr}$ and $tRNA^{Ser}$. Normal $tRNA^{Tyr}$ was eluted from Octyl-Sepharose as a single peak somewhat later than $tRNA^{Phe}$, while $tRNA^{Tyr}$ from methionine-starved *E. coli* W6 was eluted as two peaks, well separated from each other (Fig. 6). The last peak eluted identically with the normal $tRNA^{Tyr}$. The first peak eluted represents methyl-poor $tRNA^{Tyr}$. When tRNA from *E. coli* miaA was used, $tRNA^{Tyr}$ was eluted as a single peak much earlier than the methyl-poor $tRNA^{Tyr}$. This pattern for the three different types of $tRNA^{Tyr}$ with ms^2i^6A, s^2i^6A and A, respectively, at position 37 is very similar to that found for $tRNA^{Phe}$.

Chromatography of tRNA on Octyl-Sepharose resulted in a more complicated picture for $tRNA^{Ser}$ (Fig. 7). Normal $tRNA^{Ser}$ was eluted as three rather well separated peaks A–C, representing different isoaccepting species. When tRNA from methionine-starved *E. coli* W6 was used, peaks A and B appeared as for normal tRNA, but peak C was decreased by about 50%, while a new peak D appeared earlier in the chromatogram between peaks B and C. Our interpretation is that peak C represents the isoacceptor $tRNA_1^{Ser}$, which is known to contain ms^2i^6A at position 37, while peaks A and B represent other serine isoacceptors not containing ms^2i^6A. $tRNA_3^{Ser}$

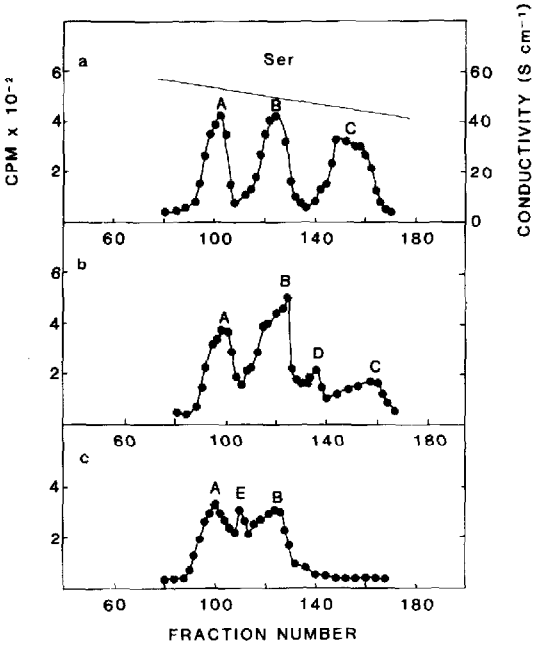

Fig. 7. Chromatography on Octyl-Sepharose of tRNA from (a) normal *E. coli*, (b) methionine-starved *E. coli* W6 and (c) *E. coli* miaA. The tRNA was postlabelled with [^{14}C]serine.

is known to contain t^6A at position 37, which modification is not affected by methionine starvation. $tRNA_2^{Ser}$ has not yet been sequenced. Peak D should then represent $tRNA_1^{Ser}$ with the incomplete modification s^2i^6A at position 37.

When *E. coli* miaA tRNA was chromatographed, peaks A and B appeared as for normal tRNA, while peaks C and D were absent. A new peak E appeared between peaks A and B. This peak should represent $tRNA_1^{Ser}$ with completely unmodified adenosine at position 37.

Interpreted in this way, the elution patterns for $tRNA^{Tyr}$ and $tRNA^{Ser}$ are fully consistent with our results from chromatography of $tRNA^{Phe}$.

DISCUSSION

Logarithmically grown *E. coli* cells contain one major and two minor species of $tRNA^{Phe}$[7]. The minor species are only faintly discernible in chromatograms, and their nucleotide sequences are still unknown. They are present in negligible amounts in the tRNA used in this investigation. Our discussion will refer to the major $tRNA^{Phe}$ only.

When a relaxed strain of *E. coli* is incubated under conditions of methionine starvation, unmethylated species of the different tRNAs accumulate. A few hours of starvation results in a tRNA that contains roughly equal amounts of unmethylated and fully methylated species. In addition, a smaller portion of the tRNA may be partially methylated. The modified nucleosides in *E. coli* $tRNA^{Phe}$ known to be derived from methionine are m^5U, m^7G, ms^2i^6A and X. These are supposed to be absent in tRNA from methionine-starved, relaxed *E. coli*. It cannot, however, be excluded that other modifications might be affected by methionine starvation.

The difference in elution from Octyl-Sepharose between normal $tRNA^{Phe}$ and methyl-deficient $tRNA^{Phe}$ has here been shown to depend on the presence and absence, respectively, of the methylthio moiety of ms^2i^6A at site 37. There is some controversy in the literature concerning this point. Isham and Stulberg[24] reported the presence of i^6A in incompletely methylated $tRNA^{Phe}$, while Agris *et al.*[25] reported only small amounts of i^6A but high amounts of a sulphur-containing derivative of i^6A that most probably is s^2i^6A. It is believed that s^2i^6A is unstable in tRNA and is easily degraded to i^6A. We cannot from our data decide whether methionine starvation in our $tRNA^{Phe}$ has resulted in s^2i^6A or i^6A. However, it is inconceivable that any hydrophobic interaction could be due to the polar thio group itself.

It is surprising that the single methyl group of ms^2i^6A has such a great effect on the hydrophobic character of $tRNA^{Phe}$ as elicited by the Octyl-Sepharose chromatograms. The effect is comparable with that of the much more hydrophobic isopentenyl group. In contrast, the methyl groups of m^5U, m^7G and m^2A do not perceptibly interact with Octyl-Sepharose (Figs. 1c and 2). One speculative explanation for this discrepancy is that these other methyl groups could be sterically unavailable for hydrophobic interaction with Octyl-Sepharose, while the methyl group of ms^2i^6A, positioned on the sulphur atom, protrudes more so as to reach the octyl groups and possibly also the methyl groups of the agarose matrix. To account for the rather strong interaction, one may speculate further that the methyl group and the nearby isopentenyl group might fold towards each other to interact hydrophobically, thereby or in another way cooperatively forming a more pronounced hydrophobic surface.

The strong hydrophobic interaction between the methyl group of site 37 and Octyl-Sepharose is also found with permanganate-oxidized $tRNA^{Phe}$. Permanganate is known to oxidize specifically the isopentenyl group of tRNA, the primary product being 2,3-dihydroxyisoamyladenosine. The final product, after an unknown sequence of intermediates, is reported to be adenosine[27]. In our experiments, the product of permanganate oxidation is probably a mixture of the intermediates, but definitely not adenosine, since all the oxidized $tRNA^{Phe}$ are eluted later than $tRNA^{Phe}$ from *E. coli* miaA. It is therefore possible that oxidized $tRNA^{Phe}$ still has a hydrophobic residue derived from the isopentenyl group, and that this residue can interact with the methyl group at the same position on tRNA.

Our results are consistent with those of Juarez *et al.*[7] who manipulated *E. coli* $tRNA^{Phe}$ in different ways and analyzed the products by elution from benzoylated DEAE-cellulose. It is evident from both their and our work that hydrophobic interaction chromatography is a sensitive and potent tool for both analysis and purification of tRNAs with varying degrees of modification.

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