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Enzymatic methylations: III. Cadaverine-induced conformational changes of E.coli $tRNA^{fMet}$ as evidenced by the availability of a specific adenosine and a specific cytidine residue for methylation.+

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ABSTRACT

A partially purified tRNA methylase fraction from rat liver, containing m^2G-, m^1A- and m^5C-methylase, was used to study the influence of Mg^{++} and of the biogenic polyamine cadaverine on the enzymatic methylation of E.coli $tRNA^{fMet}$ in vitro. In presence of 1 or 10 mM Mg^{++}, guanosine no. 27 was methylated to m^2G. In 1 mM Mg^{++} plus 30 mM cadaverine, guanosine in position 27 and adenosine in position 59 were methylated. In presence of 30 mM cadaverine alone $tRNA^{fMet}$ accepted three methyl groups: in addition to guanosine no. 27 and adenosine no. 59, cytidine no. 49 was methylated. In order to correlate $tRNA^{fMet}$ tertiary structure changes with the methylation patterns, differentiated melting curves of $tRNA^{fMet}$ were measured under the methylation conditions. It was shown that the thermodynamic stability of $tRNA^{fMet}$ tertiary structure is different in presence of Mg^{++}, or Mg^{++} plus cadaverine, or cadaverine alone. From the differentiated melting curves and from the methylation experiments one can conclude that, at 37° in the presence of Mg^{++} $tRNA^{fMet}$ has a compact structure with the extra loop and the TΨC-loop protected by tertiary structure interactions. In Mg^{++} plus cadaverine, the TΨC-loop is available, while the extra loop is yet engaged in tertiary structure (G-15 : C-49) interactions. In cadaverine alone, the TΨC-loop and the extra loop are free; hence under these conditions the open $tRNA^{fMet}$ clover leaf may be the substrate for methylation. In general, cadaverine destabilizes $tRNA^{fMet}$ tertiary structure in the presence of Mg^{++}, and stabilizes $tRNA^{fMet}$ tertiary structure in the absence of Mg^{++}. This may be explained by a competition of cadaverine with Mg^{++} for specific binding sites on the tRNA. On the basis of these experiments a possible role of biogenic polyamines in vivo may be discussed: as essential components of procaryotic and eucaryotic ribosomes they may together with ribosomal factors facilitate tRNA-ribosome binding during protein biosynthesis by opening the tRNA tertiary structure, thus making the tRNA's TΨC-loop available for interaction with the complementary sequence of the ribosomal 5S RNA.

INTRODUCTION

A biogenic polyamine was first seen and depicted in 1678 by the designer of the microscope, A. van Leeuwenhoek[1], who described crystals of organic origin which were later identified as spermine phosphate[2]. The biological significance of the biogenic polyamines is not clearly evident until now, albeit their widespread occurence and their importance for, and involvement in, many biological processes have been extensively investigated[3,4]. Biogenic amines have been identified as components of procaryotic and eucaryotic ribosomes[5,6,7], and their effects on reactions of nucleic acids in vitro have been described: they stimulate mRNA synthesis[8], aminoacylation of tRNA[9,10] and tRNA methylation[11,12,13]. Polyamines have been found associated with tRNA if the tRNA preparation had been performed at low ionic strength[14].

MATERIALS AND METHODS

General: (^{14}C-CH_3)-S-Adenosylmethionine was obtained from NEN Chemicals GmbH. and brought to a specific activity of 13.9 ci/M. DEAE-Sephadex A-25 was supplied by Pharmacia, 5-methyl-cytidine and 1-methyl-adenosine by Cyclo Chemical. ApApApA was from Boehringer Mannheim GmbH., hydroxyapatite HTP and Bio-Gel P-2 were from Bio-Rad Laboratories. Cadaverine (1,5-diamino-pentane) dihydrochloride was obtained from Fluka AG. n-butylamine was from Merck AG., Darmstadt. N^2-methyl- and N^2-dimethyl-guanosine were from Dr. M. Saneyoshi, Tokyo.

tRNA and oligonucleotides: All operations were performed at room temperature. Pure E.coli $tRNA^{fMet}$ was prepared from unfractionated tRNA by chromatography on DEAE-Sephadex A-50[15], followed by fractionation on benzoylated DEAE-cellulose[16]. Oligonucleotides obtained by pancreatic or T_1 RNase digestion of $tRNA^{fMet}$ or ^{14}C-methylated $tRNA^{fMet}$ were fractionated on DEAE-Sephadex A-25 in the presence of 7 m urea[17].

Enzymes: RNase T_1 (E.C.2.7.7.26) was obtained from Sankyo, Tokyo; RNase T_2 (E.C.2.7.7.17) was from Calbiochem. Pancreatic RNase (E.C.2.7.7.16), snake venom phosphodiesterase (E.C.3.1.4.1), alkaline phospatase from calf intestine (E.C.3.1.3.1) and calf spleen phosphodiesterase (E.C.3.1.4.1) were supplied by Boehringer Mannheim GmbH. Standard procedures were used for the digestion of tRNA or oligonucleotides with the above enzymes. tRNA methylases were fractionated on hydroxyapatite as described earlier[18,19,20] (Fig. 1).

Thinlayer chromatography: Precoated cellulose plates without fluorescence indicator were from Merck AG., Darmstadt. The following solvents were used: solvent A: 1-butanol : H_2O : 15 n NH_4OH (86 : 14 : 5), B: 2-propanol : H_2O : 15 n NH_4OH (7 : 2 : 1), and C: 1-propanol : H_2O : 15 n NH_4OH (55 : 35 : 10). UV absorbing spots on TLC plates were located under a 254 nm UV lamp; radioactive material on TLC plates was located by scraping the layers into scintillation vials with a half-automatic scraper[21] and counting the radioactivity in

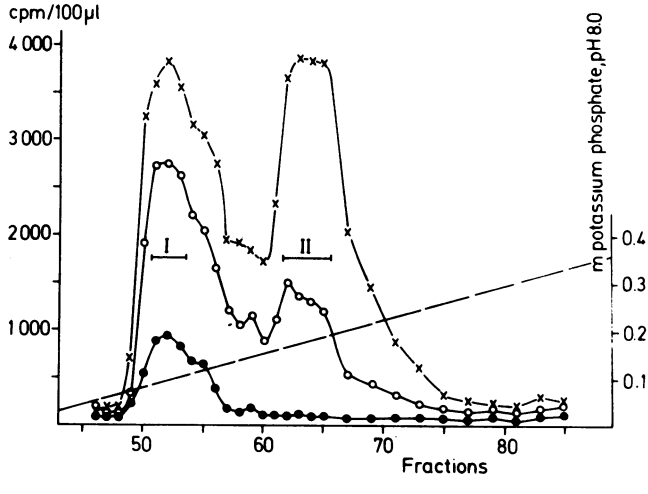

Fig.1. Fractionation of rat liver methylases on hydroxyapatite. x——x assay for tRNA methylase activity in 30 mM cadaverine (E.coli $tRNA^{fMet}$ as methyl acceptor). o——o assay for tRNA methylase activity in 10 mM Mg^{++} (E.coli $tRNA^{fMet}$ as methyl acceptor). ●——● assay for protein methylation in 30 mM cadaverine or 10 mM Mg^{++} (no significant difference; tRNA was omitted).

scintillation liquid. Radioactivity from TLC plates or from column fractions was measured in 10 ml toluene: cellulose: PPO (1000 ml : 500 ml : 6g) in a Packard Tricarb Model 3380.

Methylation of E.coli $tRNA^{fMet}$ in vitro was performed with a partially purified tRNA methylase fraction II from rat liver (Fig. 1) as described earlier[18,19,20]. The amounts of magnesium and cadaverine used for tRNA methylation are indicated in the legends to Fig. 2. 0.5 A_{260} units of purified $tRNA^{fMet}_{E.coli}$ were methylated for two hours at 35°C[18]. The material was then put on a 0.6 x 2 cm DEAE-cellulose column equilibrated with 0.02 M sodium acetate pH 5.0 and washed with 0.2 M NaCl in 0.02 M sodium acetate, pH 5.0. This step removed excess ^{14}C-S-adenosylmethionine. ^{14}C-Methylated $tRNA^{fMet}$ was eluted together with some protein material with 1 M NaCl in the above buffer. The solution was deproteinized by twofold phenolization. Physical integrity of this tRNA fraction was checked by polyacrylamide gel electrophoresis in 6 m urea[22]. To the phenolized aqueous layer 55 A_{260} units of $tRNA^{fMet}$ were added, and tRNA was precipitated by addition of 2.5 vol. of ethanol. This tRNA preparation was used for RNase T_1 and pancreatic RNase digests (Fig. 2). Oligonucleotides were desalted by filtration through a 1.5 x 50 cm Bio-Gel P-2 column[23] or by adsorbtion to 0.6 x 1 cm DEAE cellulose columns, elution of salt and urea with 50 ml 0,01 M triethylammonium bicarbonate and elution of the nucleotide material with 1 M triethyl-ammonium bicarbonate pH 8.5.

Characterization of ^{14}C-methylated oligonucleotides: m^1A in methylated $tRNA^{fMet}$ was rearranged to m^6A by controlled alkali treatment[24] prior to RNase digestion (legend to Fig. 2).

Differentiated melting curves: The differential absorption technique which has been described previously[25] was applied for the determination of the melting curves. In addition to measurements at 260 and 280 nm the absorption and hypochromicity of the 4-thiouracil base at 335 nm has been measured. The wavelength dependence of the hypochromicity is represented by the ratio $\Delta A_{260\ nm}/\Delta A_{280nm}$. All melting curves presented in Figs. 5, 6 and 7 refer to a concentration equivalent to 1 absorption unit at 260 nm, 1 cm optical path length and 20°C.

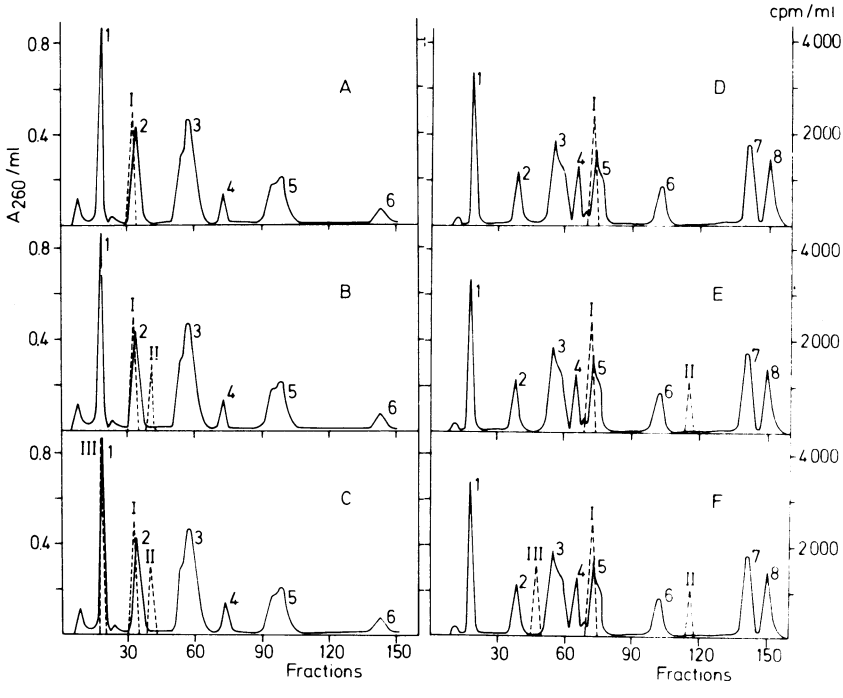

Fig.2. Chromatography of ^{14}C-methylated fragments of E.coli tRNA^{fMet} on DEAE-cellulose in 7 M urea. ——— A_{260} per ml; ---- cpm per ml. A,B,C: fragments obtained with pancreatic RNase; D,E,F: fragments obtained with RNase T_1. A,D: methylation in 1 or 10 mM Mg^{++}; B,E: methylation in 1 mM Mg^{++} plus 30 mM cadaverine; C,F: methylation in 30 mM cadaverine. The nucleotide sequences of the tRNA^{fMet} fragments are given in ref. 26 or 24 can be deduced from Fig. 4. After controlled alkali treatment (m^1A to m^6A rearrangement) prior to RNase digestion of tRNA^{fMet}, the radioactive peak II in Figs. B and C coincided with peak 4 (A-A-A-U-), and the radioactive peak II in Figs. E and F coincided with peak 7 (T-Ψ-C-decanucleotide).

RESULTS

I. Heterologous methylation of $tRNA^{fMet}_{E.coli}$

(a) Methylation of tRNA^{fMet} in 1 mM or 10 mM Mg^{++}: degradation of ^{14}C-methyl-tRNA^{fMet} with RNase A gave a single ^{14}C-methyl labeled oligonucleotide in the dinucleotide region (Fig. 2A, I), while digestion with RNase T_1 gave a single labeled oligonucleotide in the tetranucleotide region (Fig. 2D, I). Degradation of both oligonucleotides with RNase T_2 and phosphatase gave a labeled nucleoside which was identical with authentic m^2G upon cochromatography on cellulose thinlayers in

several solvent systems (as an example see Fig. 3B). There are only two tetranucleotides in a RNase T_1-digest of $tRNA^{fMet}$, C-C-U-G- and C-U-C-G-. Only C-U-C-G- is followed by a pyrimidine nucleotide, and methylation of this guanosine only yields a methylated dinucleotide upon degradation with RNase A. Hence the guanosine residue in position 27 from this tRNA's 5'-end is the site of methylation (Fig. 4).

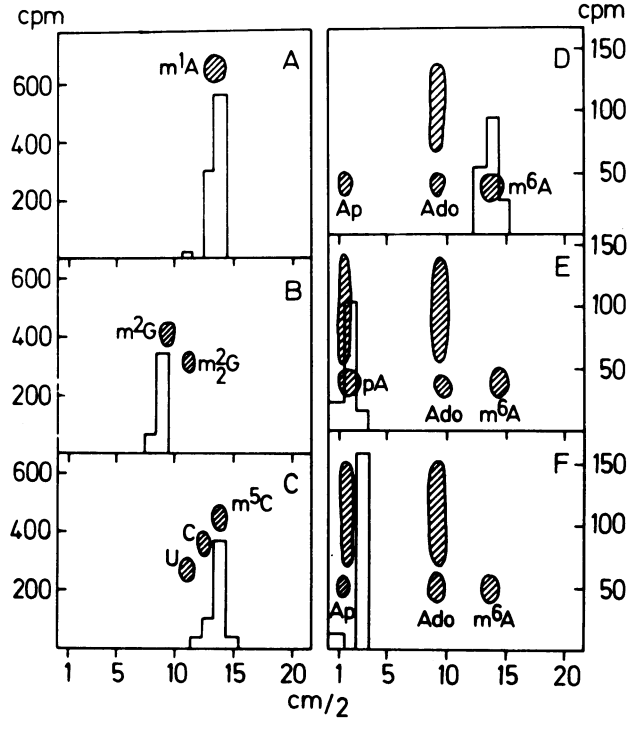

Fig. 3. Thinlayer chromatography on cellulose (solvent B). A,B, C: Authentic samples (shaded spots) were compared with ^{14}C-methylated nucleosides from E.coli $tRNA^{fMet}$ (white blocks, cpm per 0.5 cm). D,E,F: Thinlayer chromatography of nucleosides and nucleotides obtained from m^6(A-A-A) after digestion with RNase T_2 and bacterial alkaline phosphatase (D), after digestion with snake venom phosphodiesterase (E) and after degradation with spleen phosphodiesterase (F).

(b) $tRNA^{fMet}$ methylated in 1 mM Mg^{++} and 30 mM cadaverine: in addition to the methylation of guanosine no. 27, a second methylated oligonucleotide (peak II, Figs. 2B;2E) was observed. Upon RNase T_1 degradation it was eluted between the hexanucleotide and the decanucleotide region (Fig. 2E). After digestion

with RNase A a new methylated oligonucleotide (peak II, Fig.2B) was eluted between the di- and trinucleotides. Degradation of both radioactive oligonucleotides with RNase T_2 and phosphatase gave a ^{14}C-methyl nucleoside which was identical with authentic m^1A in thinlayer chromatograms using different solvent systems (as an example see Fig. 3A). In order to find the position of m^1A in the $tRNA^{fMet}$ sequence, we treated an aliquot of the methylated tRNA from above with alkali under conditions which rearrange m^1A to m^6A[24]. This rearrangement causes loss of the positive charge of m^1A and therefore changes the elution of the corresponding oligonucleotides from the DEAE-column. After this rearrangement and RNase T_1 digestion the methylated oligonucleotide coincided with the T-Ψ-C-A-A-A-U-C-C-G- decanucleotide (Fig. 2E, peak 7), while the corresponding RNase A-fragment now cochromatographed with the tetranucleotide A-A-A-U- (Fig. 2B, peak 4). This shows that one adenosine residue in the A-A-A triplet of the TΨC-loop (Fig. 4) was methylated. The position of the methylated adenosine was found as follows: the tetranucleotide A-A-A-U- was pooled together with the rearranged m^6(A-A-A)-U-, and after dephosphorylation with phosphatase, 5 A_{260} units of the commercial tetranucleoside triphosphate A-A-A-A were added. The mixture was oxidized with periodate and treated with cyclohexylamine at 45°C[27]. The resulting material was desalted, treated with phosphatase and purified by TLC. The previously added A-A-A-A had disappeared as shown by an authentic marker on the same TLC plate, and a faster moving band appeared, indicating degradation of A-A-A-A to A-A-A in a very high yield. The radioactive trinucleoside diphosphate m^6(A-A-A) was located with a TLC scanner just ahead of A-A-A. The A-A-A band and the labeled m^6(A-A-A) band were scraped out together and the oligonucleotide material was isolated by extraction. An aliquot was digested with spleen phosphodiesterase, while another aliquot was digested with snake venom phosphodiesterase.

The digests were chromatographed on cellulose thinlayers in solvent B, which efficiently separates nucleosides and mononucleotides. In both digests the radioactivity was detected only in the mononucleotide region of the TLCs (m^6Ap

and pm^{6}A, Fig. 3E and 3F). No radioactivity was detected in the added m^{6}A marker. Treatment of ^{14}C-methyl m^6(A-A-A) however with snake venom phosphodiesterase plus phosphatase gave a radioactive nucleoside identical with authentic m^{6}A in the above TLC (Fig. 3D). This shows that only the central adenosine, A-59, in the TΨC-loop's A-A-A sequence had been methylated (Fig. 4).

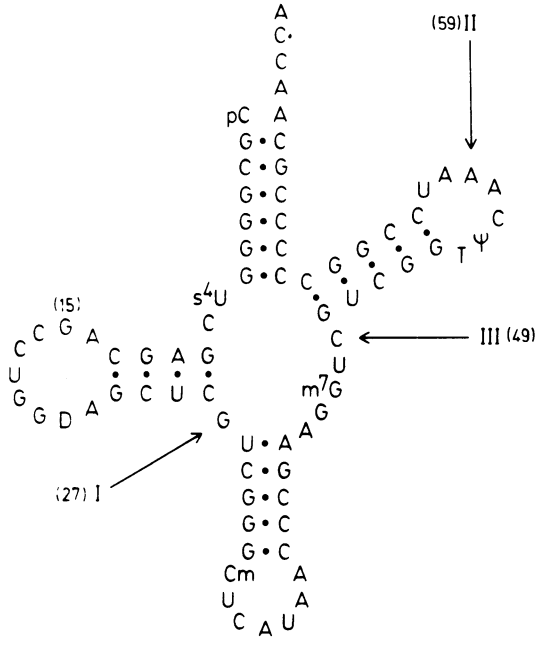

Fig. 4. Nucleotide sequence[28] of E.coli tRNA^{fMet}. No tRNA^{fMet} with an internal cross-link [29] was present in our preparation, which was a 3:1 mixture of the major and the minor tRNA^{fMet} species[30,31].- The arrows point onto the sites of methylation with rat liver methylase.I: m^{2}G in position 27 from the 5'-end is obtained in presence of Mg^{++}. II: in presence of Mg^{++} plus cadaverine, m^{1}A is found in position 59 in addition to m^{2}G(I). III: in presence of cadaverine alone, m^{5}C is formed in position 49 in addition to m^{2}G (I) and m^{1}A (II).

(c) tRNA^{fMet} methylation in 30 mM cadaverine: besides the two methylated oligonucleotides described above, a third peak of radioactivity was observed. After RNase T_1 digestion a new radioactive peak (III, Fig. 2F) was located between the di- and trinucleotides, while there was an additional radioactive

peak in the mononucleotide region of the pancreatic digest (peak III, Fig. 2C). Degradation of both peaks III to the nucleosides gave a ^{14}C-methyl nucleoside which was found to be identical with authentic m^5C in TLC in several solvents (as an example see Fig. 3C).

The elution of peaks III in Figs. 2C and 2F indicates, that the newly formed m^5C must be located in a sequence pyr-m^5C-G- (pyr = pyrimidine nucleotide). There are three such sequences in $tRNA^{fMet}$: U-C-G- in the anticodon stem, U-C-G- in the T-Ψ-C stem, and m^7G-U-C-G- in the extra loop. We treated peak III from the T_1 digest (Fig. 2F) with alkali under conditions which cause cleavage of m^7G to a 4-ribosylamino pyrimidine derivative lacking the positive charge of m^7G [32].

Rechromatography under the conditions of Fig. 2F showed, that the m^5C-containing oligonucleotide now cochromatographed with the tetranucleotide fraction (peak 4, Fig. 2F). This allows to conclude that only C-49 in the m^7G-C-U-G- sequence in the extra loop is the site of methylation in the presence of 30 mM cadaverine (Fig. 4).

Stimulation of $tRNA^{fMet}$ methylation in the absence of Mg^{++} with different amines: optimal stimulation was achieved in the presence of 0.1 mM spermidine, 0.05 mM spermine, 30 mM putrescine and 30 mM cadaverine. The latter was used in all methylation experiments and for measuring differentiated $tRNA^{fMet}$ melting curves under corresponding conditions. In contrast to the above biogenic amines, no stimulation of $tRNA^{fMet}$ methylation was observed in the presence of 30 mM n-butylamine. Non-aliphatic biogenic amines like dopamine, noradrenaline,and tyramine (30 mM) had no stimulating effect at all.

II. Thermodynamic measurements

Melting curves have been carried out to correlate the different degrees of methylation to the thermodynamic stabilities of different parts of the tRNA molecule.

In the absence of cadaverine. First it is shown that the thermal denaturation process of $tRNA^{fMet}_{E.coli}$ in the absence of cadaverine is in accordance with the results known for this [33] and other systems [34]. Under conditions of medium ionic strength (10 mM sodium-cacodylate, 100 mM NaCl) and in the absence of

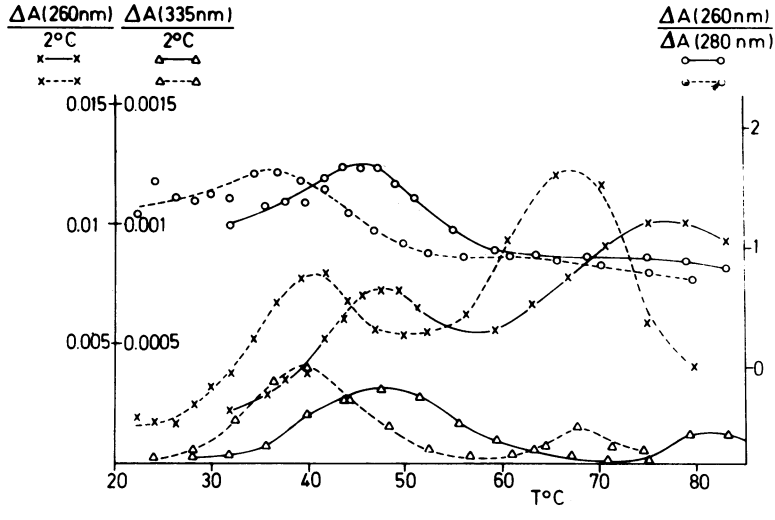

Fig.5. Differentiated melting curves of E.coli tRNA^{fMet} in 10 mM sodium cacodylate - 100 mM NaCl, pH 6.8. ---- no cadaverine added; ——— in the presence of 30 mM cadaverine.

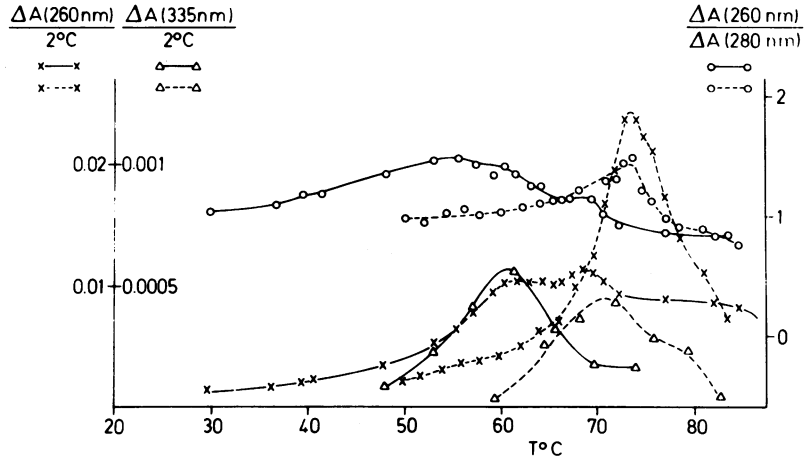

Fig.6. Differentiated melting curves of E.coli tRNA^{fMet} in 100 mM Tris-HCl pH 7.5, 1 mM Mg^{++}. ---- no cadaverine added; ——— in the presence of 30 mM cadaverine.

Mg^{++}-ions the melting is clearly biphasic (Fig. 5). The peak at lower temperature is called "early melting". It has been shown by kinetic experiments[33], that two processes with somewhat different stabilities contribute to the low temperature peak of the melting curve. In general, all or none - processes can be described spectroscopically by the wavelength dependence of the hypochromicity which is represented in our measurements by the ratio ΔA (260 nm)/ΔA (280 nm). Fig. 5 shows that this ratio is not constant over the low temperature peak, because of the contribution of two transitions in accordance with the kinetic results. The mean values of the ratios ΔA (260 nm)/ΔA (280 nm) are significantly higher in the low temperature transitions (1.3 - 1.6) than in the high temperature part of the melting curve and can be used to identify experimentally those processes under varying conditions.

In all tRNAs investigated so far, the low temperature peak is more stabilized by Na^{+} or Mg^{++} ions (in the absence of cadaverine) than the high temperature peak. This has been checked also for $tRNA^{fMet}$ (E.coli) and is evident from a comparison of the corresponding melting curves in Figs. 5 and 6 (dotted curves). The two peaks which are resolved in the absence of Mg^{++} are superimposed on the temperature scale, if Mg^{++} is present. The melting curve in Fig. 6 (dotted curve) corresponds to the conditions (a) in the methylation experiments.

Influence of cadaverine. When Mg^{++} is absent, cadaverine shifts both maxima to higher temperatures (Fig. 5). Otherwise, the melting curves followed at 260, 280 and 335 nm with and without cadaverine are very similar. Cadaverine induces no preferential stabilization of the low temperature melting peak, as Mg^{++}-ions do; rather it shifts more the maximum of the high temperature-region. This effect is most obvious in low ionic strength (Fig. 7) under conditions (c) of the methylation experiments.

Of particular interest is the influence of 30 mM cadaverine in the presence of 1 mM Mg^{++} (corresponding to methylation experiment (b)). Cadaverine destabilizes a part of the structure as is evident from the melting curves in Fig. 6. This destabilized transition is connected with the high ratio ΔA (260 nm)/ΔA (280 nm) and the main contribution to the

hypochromicity at 335 nm.

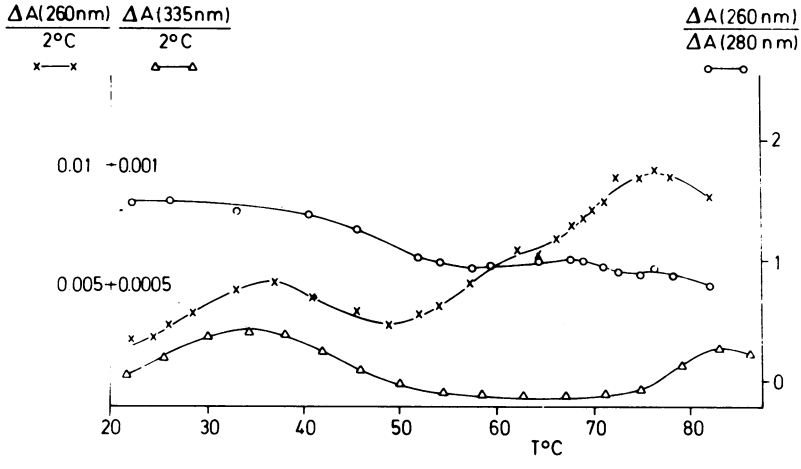

Fig. 7. Differentiated melting curves of E.coli $tRNA^{fMet}$ in 100 mM Tris-HCl pH 7.5, 30 mM cadaverine.

DISCUSSION

The stimulatory effect of polyamines on tRNA methylation was first reported by Leboy[11]. It was not clear whether this effect, which was observed also by other authors[12,13] was due to an increased methylation velocity or due to an altered specificity of tRNA methylases, or due to the availability of new sites for methylation. Leboy[35] and Pegg[36] could exclude the first possibility since they demonstrated the occurence of new methylated nucleosides after tRNA methylation in the presence of polyamines compared to methylation in the presence of magnesium. A tRNA tertiary structure-dependent stepwise increased availability of sites for tRNA methylation, however, as we report here, had not been demonstrated before. For our experiments we first assayed HAP column fractions of a rat liver supernatant[18,19] for those methylase fractions with maximal stimulation by biogenic amine (Fig.1). Both peaks of methylase activity gave identical methylation patterns with $tRNA^{fMet}_{E.coli}$, the minimum of methylase activity between both peaks I and II most likely being caused by an S-adenosyl-methionine degrading enzyme[37]. Methylase fraction II was used for our experiments because of the better stimulation with

biogenic amine and because of low nuclease contamination. As shown above, these methylases are of different specifity, m^2G-methylase, m^1A - methylase probably in lower concentration, and m^5C - methylase. The presence of only small amounts of m^1A methylase in fraction II had also been observed previously[19]. In our experiments we have not intended to obtain 100 % m^1A formation (by longer incubation or higher enzyme levels) in order to avoid any nuclease action on $tRNA^{fMet}$. It was extremely important to show that the methylation patterns obtained were not caused by a conformational change due to limited nuclease degradation, since Kuchino et al.[19] had found different methylation patterns for intact $tRNA^{fMet}_{E.coli}$ and a combination of two large $tRNA^{fMet}$ fragments. We have the following criteria for the integrity of $tRNA^{fMet}$ under our methylation conditions: (a) only intact ^{14}C-methylated pancreatic and RNase T_1 fragments were observed (b) kinetics and methylation were measured up to 125 min. and in all cases came to plateaux. The presence of very significant amounts of nucleases would have caused the plateaux to decline; (c) aliquots of methylated $tRNA^{fMet}$ were run in polyacrylamide electrophoresis in 6 M urea[22] showing that $tRNA^{fMet}$ degradation did not occur under our conditions. Hence nuclease action is not responsible for the different levels of $tRNA^{fMet}$ methylation. We therefore tried to demonstrate the existence of conformational differences of $tRNA^{fMet}$ in 1 mM Mg^{++}, 1 mM Mg^{++} plus 30 mM cadaverine and in 30 mM cadaverine without Mg^{++}, and to correlate the possible differences with our methylation patterns under corresponding conditions.

Conformational transitions in tRNA are understood to some extent from thermodynamic experiments. It is possible to differentiate between conformational transitions, which represent opening of tertiary structure and double-helix-coil transitions of the different cloverleaf branches. The general scheme is outlined in detail elsewhere[34]. The early melting in $tRNA^{fMet}_{E.coli}$ was experimentally characterized by several findings: high ratio ΔA (260 nm)/ ΔA (280 nm), main contribution to the hypochromicity at 335 nm,

strong influence of Mg^{++}-ions on the thermal stability, and separation in two kinetically resolvable steps. This process was interpreted[33] as the opening of tertiary structure involving most probably also the opening of the dihydrouridine branch. Unfolding of tertiary structure in a low temperature melting step was also found in other tRNA species[38,39]. It was now possible to study the influence of cadaverine on the thermodynamic stability of the tertiary structure and to check whether the tertiary structure can be correlated to the methylation pattern.

In the presence of 1 mM (or 10 mM) Mg^{++} only one methyl group is incorporated into G-27 and no significant melting effect below 50°C was observed. It is generally accepted, that at 37°C and in 1 mM Mg^{++} the tertiary structure is completely stable. Furthermore the structure has to be quite compact in solution since no m^1A or m^5C were formed under these conditions. As far as methylation is concerned the T-Ψ-C-loop and the extra loop are blocked by tertiary structure interactions. This finding is in agreement with results obtained with other methods e.g. oligonucleotide binding[40], chemical modification[41] and partial enzymatic digestion[42].

In the presence of cadaverine alone A-59 and C-49 are accessible to methylation in addition to G-27; these are the sites of methylation in vivo of mammalian cytoplasmic initiator tRNAs[43,44]. Since under our conditions the earlier melting process has its midpoint temperature at 40°C, the tertiary structure has unfolded in nearly one half of all molecules. Taking into account that the early melting process occurs in two steps, one has to specify that at 40°C the molecules have undergone the first step to a higher degree than the second step. Both of the processes are in a reversible thermodynamic equilibrium and therefore all sites, which are accessible when both unfolding steps have occured, can be methylated. The form active as acceptor for methylation at G-27, A-59 and C-49 is most probably the open clover leaf with tertiary structure mainly unfolded.

The discussion of the results obtained in presence of 1 mM Mg^{++} and 30 mM cadaverine is more involved. The methylation experiments showed that addition of cadaverine induces

accessibility of A-59 in the T-Ψ-C-loop, whereas C-49 in the extra-arm remains yet protected.
The T_m-value of the tertiary structure melting is lowered by more than 10°C. In this respect both types of experiments indicate that in the presence of Mg^{++}, cadaverine can induce an opening - at least partially - of the tertiary structure.

The T_m value however is still about 20°C higher than the temperature of the methylation experiments; that means only a small percentage of the tRNA molecules is in the state of the unfolded tertiary structure. If tertiary structure opens up in two steps, it is most probable that, only the first one may have occured under methylation conditions. Accessibility of A-59 indicates that those parts of the tertiary structure which involve the TΨC-loop open before those involving C-49. A G-15: C-49 interaction has been postulated in the Levitt-model for tRNA[45], was confirmed by chemical reactions [46] and is in accordance with the recent x-ray results on $tRNA^{Phe}_{yeast}$ [47,48]. Hence it is reasonable to assume that, under our conditions, the interaction between G-15 and C-49 is associated with the most stable part of $tRNA^{fMet}_{E.coli}$ tertiary structure.
Since cadaverine destabilizes the tertiary structure only in the presence of Mg^{++}-ions, the effect is most probably due to a competition of cadaverine and Mg^{++} for a particular class of binding sites on the tRNA. The Mg^{++}-ions, which stabilize most effectively tertiary structure are expelled by an excess of the cadaverine ions, which stabilize tertiary structure much less. Therefore in a particular concentration range, the net effect of the addition of cadaverine is a destabilization of the tertiary structure. Similar antagonistic effects had been observed on DNA between Mg^{++} and monovalent ions[49].-

It should be mentioned here that three methyl groups (as described here, fig. 4) have been introduced into E.coli $tRNA^{fMet}$ with a crude methylase from rat hepatoma[50] in the presence of 0.4 M ammonium acetate[51], and that this threefold hypermethylated tRNA had the same acceptor activity as native $tRNA^{fMet}$ [52].-
The influence of cadaverine (and possibly of biogenic polyamines in general) on $tRNA^{fMet}$ tertiary structure and methylation may

be generalized as follows: cadaverine (or other polyamines) acts as an allosteric effector onto the "allosteric" substrate $tRNA^{fMet}$, thus altering its conformation and hence its reactivity. Our experiments provide evidence that, besides primary and secondary structure, tertiary structure is of special importance for $tRNA^{fMet}$ methylation in vitro. There is the possibility, however, that biogenic polyamines are not involved in tRNA methylase regulation in vivo[53].-

It is discussed, that under our conditions the TΨC-loop is opened before the G-15 : C-49-interaction is lost. This means that biogenic amines (at least cadaverine), in the presence of Mg^{++}, can make available the TΨC-loop at a physiological temperature without disrupting all of the tRNA tertiary structure.

One might be tempted to speculate that the biological function of biogenic polyamines, which are essential components of procaryotic and eucaryotic ribosomes[5,6,7], might be to facilitate, together with ribosomal factors, the interaction of the TΨC ribosome binding site of the tRNA[54,55,56] with the ribosomal surface, i.e. with a complementary sequence of the ribosomal 5S RNA[57,58]. The enhancement of N-acetyl-phenylalanyl-tRNA binding to E.coli ribosomes by biogenic polyamines at low Mg^{++} concentrations has been observed recently[59].

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