4-THIOURIDINE AND THE CONFORMATION OF E. COLI tRNA INDUCED BY SPERMIDINE

F. Pochon* and S. S. Cohen**

Department of Microbiology, University of Colorado Medical Center
Denver, Colorado 80220

Received April 10, 1972

SUMMARY: The reactivity of 4-thiouridine in E. coli tRNA is affected in several ways by spermidine and by Mg^{++}. Spermidine increases the fluorescence of this nucleoside in tRNA about half as much as does Mg^{++}. At low ratios of cation to tRNA, the two ions together are more active than either ion alone. The rate of formation of a photoproduct linking thiouridine (position 8) and nonadjacent cytidine (position 13) is greater in the presence of spermidine than with Mg^{++}. The fluorescence of the $NaBH_4$-reduced photoproduct made in Mg^{++} is reduced by dialysis and then more effectively restored by spermidine than by Mg^{++}. Spermidine appears to effect a conformational change involving the contiguous dihydrouridine and C·C·A arms of tRNA.

Spermidine as well as Mg^{++} have been demonstrated to stimulate numerous reactions of transcription and translation (1). Among these are reactions with tRNA (2,3), as in the transfer of an amino acid from an isolated enzyme - amino acid - adenylate complex to acylate tRNA (4). Recently it was shown that the fluorescence of the Y base in the anticodon arm of yeast phenylalanyl tRNA is increased by spermidine and the amino acid acceptance of preparations of this tRNA is increased in a parallel fashion (5). Leboy has described the activation of a liver tRNA methylase by polyamine, and most recently has indicated significant differences between methylations stimulated by spermidine or Mg^{++} (6). Spermidine can evidently help to organize active conformations of tRNA at apparently physiological concentrations of the natural bacterial polyamine.

When tRNA is isolated at low ionic strength from E. coli harvested during the synthesis of both tRNA and spermidine, the tRNA is found to contain 2 moles

---

*Present address: Institut de Biologie Physico-Chimique, 13 Rue Pierre Curie, Paris, France.
**To whom correspondence should be addressed. This work was supported by a USPHS grant AI 10424-01.

of spermidine per mole (7). In recent studies (C. Freda and S. S. Cohen - in preparation) it has been observed that tRNA contains 2 to 3 relatively tight binding sites and 7-8 relatively weak binding sites for spermidine. We are attempting to localize these tight binding sites of E. coli tRNA and have asked if the arm of tRNA containing dihydrouracil (DHU), which contains only three or four complementary base pairs (depending on the specific tRNA), uses spermidine to stabilize this limited helical structure. We have studied several activities of 4-thiouridine (Srd) (position 8) which is at the juncture of the DHU and CCA arms of the nucleic acid. These include a comparison of spermidine and Mg^{++} in 1) enhancing the fluorescence of Srd in tRNA (8), 2) enhancing the rate of formation of the photoproduct (9) formed between the nonadjacent nucleosides, Srd and cytidine (position 13), and 3) enhancing the fluorescence of the preformed photoproduct (Srd-Cyt) reduced by $NaBH_4$. The formation of the photoproduct and its reduction are described in Figure 1.

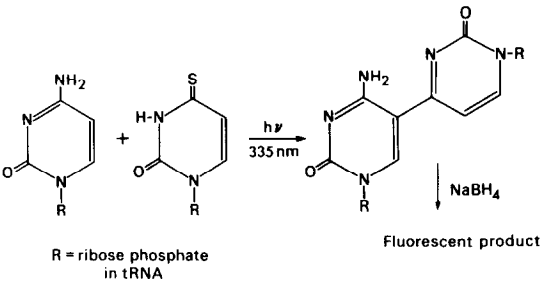

Figure 1: Photoproduction of 5-(4'-pyrimidin-2'-one) cytosine in E. coli tRNA and further reduction to a fluorescent derivative (10).

The functional conformation of a closely packed tertiary structure in tRNA appears to be possible when limited numbers of divalent cations neutralize the negatively charged phosphates. In many species of E. coli tRNA, the fluorescence of Srd at position 8 is determined by the tertiary conformation, e.g. by proximity to nucleosides noncontiguous in the primary structure. Unfractionated, unstripped E. coli tRNA (Calbiochem and General Biochemicals) was dialysed extensively (2 days at 6° at each step) against 0.2 M NaCl containing

0.01 M EDTA pH 7.0, and against water. Hydrochlorides of spermidine, spermine and of putrescine, estimated to be pure by titration, and devoid of contaminating polyamine by the dansyl method (7), or $MgCl_2$ were added to the tRNA. Fluorescence of the tRNA in K acetate 0.01 M pH 7.0 (0.038 µmole tRNA per ml, derived from an extinction coefficient at 260 nm of 570 per µmole/ml/cm) was estimated in an Aminco-Bowman fluorimeter.

As shown in Figure 2, addition of Mg^{++} to a ratio of 13 to 14 moles per mole of tRNA gives a greater than three fold enhancement of fluorescence of Srd.

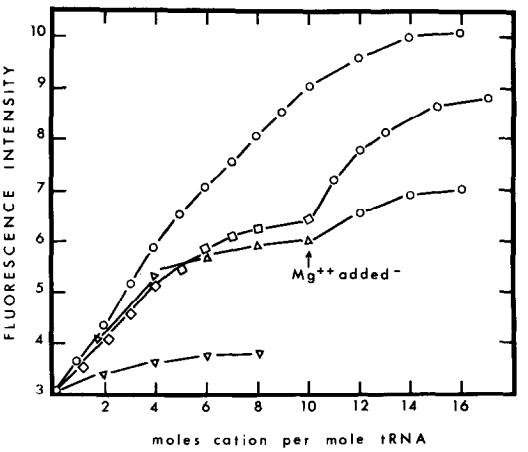

Figure 2: Effect of cations on the intensity of fluorescence of Srd in E. coli tRNA. Samples of tRNA (0.052 µmole) in 1.5 ml K acetate 0.01 M pH 7.0 were titrated with $MgCl_2$ 0---0, and spermidine □--□, spermine Δ---Δ and putrescine ∇---∇, and the emission at 530 nm was measured after excitation at 335 nm. Fluorescence is expressed in arbitrary units; temperature = 22°.

On the other hand the addition of spermidine produces its maximum effect of doubling the basal fluorescence at a ratio of 10 moles per mole. Fluorescence is increased similarly but slightly less by spermine, and very much less by putrescine. Addition of Mg^{++} to the spermidine system increased fluorescence to about a three fold enhancement, significantly less than that produced by Mg^{++} alone. Mg^{++} scarcely enhanced the fluorescence of the spermine system. The four cations produce very different conformations of tRNA, as estimated by this parameter.

High concentrations of Mg^{++} do not totally displace spermidine from a spermidine-tRNA complex (10 moles per mole). After extensive dialysis against 0.02 M Mg^{++}, 0.01 M K acetate, the dansyl procedure revealed 2 moles of residual spermidine per mole.

When small increments of spermidine are added to tRNA titrated with 2, 4, or 6 moles of Mg^{++} per mole, as shown in Figure 3 the fluorescence of the system is markedly increased compared with that achieved by Mg^{++} or spermidine alone at the same ratios of cation per mole of tRNA. Thus the two cations when added in this sequence synergize the formation of a conformation in which the fluorescence of Srd is more readily expressed. A maximum fluorescence appears to occur cooperatively in the sequence of 2 atoms of Mg^{++}, 8 molecules of spermidine, and finally 2 atoms of Mg^{++} per mole of tRNA.

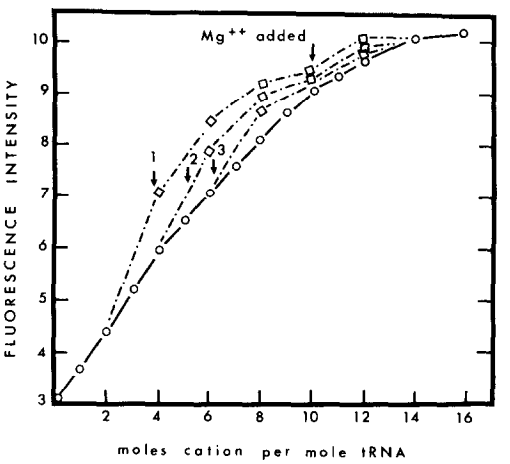

Figure 3: Effect of cations on the fluorescence intensity of Srd in E. coli tRNA. Samples of tRNA (0.052 µmole) in 1.5 ml K acetate 0.01 M pH 7.0 were titrated with $MgCl_2$ 0---0, and spermidine was added at intermediate contents of Mg^{++}. Curve 1 - 0.1 µmole Mg^{++}, followed by spermidine (0.4 µmole total); Curve 2 - 0.2 µmole Mg^{++} followed by spermidine (0.3 µmole total); Curve 3 - 0.3 µmole Mg^{++} followed by spermidine (0.2 µmole total). At the plateaus of fluorescence established by spermidine, Mg^{++} was again added. Excitation at 335 nm and emission at 530 nm. Fluorescence in arbitrary units; temperature = 22°.

It has been reported that the formation of a Srd-Cyt photoproduct requires Mg^{++} (8,9) or a high concentration of NaCl (0.1 M); many preparations of the latter salt contain significant amounts of Mg^{++}. Irradiation at 350 nm in 0.1

M NaCl, 0.001 M Mg^{++}, 0.05 M in sodium cacodylate pH 7.0, or in the buffer mixture comprised of 0.01 M K acetate, pH 7.0 in the presence of Mg^{++} or spermidine or mixtures of these cations gives rise to the same Srd-Cyt photoproduct (10). This was shown by the disappearance of fluorescence of Srd, and by parallel increases of optical density at 360 nm and the fluorescence of the Srd-Cyt reduced by $NaBH_4$. In the experimental conditions described in the Legend to Figure 4, only the initial rate of formation of Srd-Cyt is modified. Irradiation for 15 hours produces the same extent of photoreaction in all instances tested.

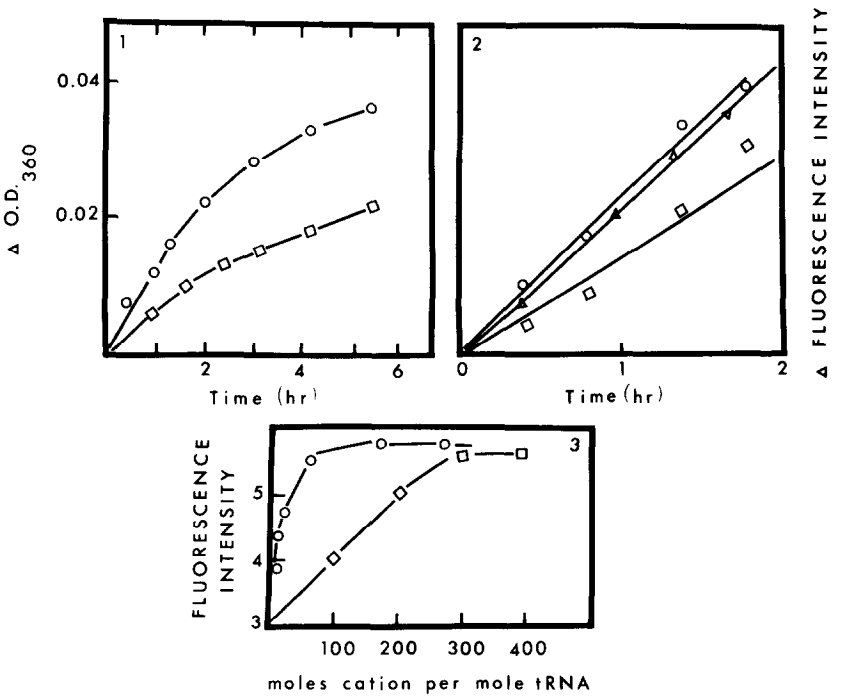

Figure 4: Initial rate of formation of the photoproduct Srd-Cyt in E. coli tRNA.
1. Increase of ultraviolet absorption at 360 nm after irradiation at 350 nm. Solution of tRNA (0.053 µmole) in 2 ml K acetate 0.01 M pH 7.0 with 0.4 µmole Mg^{++} □---□ and 0.4 mole of spermidine 0---0.
2. Increase of fluorescence of a solution of tRNA (0.053 µmole in 3 ml K acetate 0.01 M pH 7.0) after irradiation with 0.1 µmole Mg^{++} + 0.3 µmole spermidine 0---0, with 0.4 µmole Mg^{++} □---□, and with 0.4 µmole spermidine Δ---Δ followed by reduction by $NaBH_4$. Excitation = 400 nm; Emission = 450 nm. Fluorescence in arbitrary units; temperature = 22°.
3. Effect of cations on the fluorescence of reduced Srd-Cyt in E. coli tRNA. Samples (2.0 ml) of tRNA (0.0012 µmole) dialysed against EDTA and K acetate 0.01 M pH 7.0 are titrated with Mg^{++} □---□, and spermidine 0---0. Excitation 400 nm, emission 450 nm. Fluorescence in arbitrary units; temperature = 22°.

Spermidine is more effective than Mg^{++} at the same concentration with respect to the initial rate of the photoreaction (Fig. 4 - No. 1), even though the organic cation produces a lower increment of fluorescence of Srd. Apparently the presence of spermidine organizes the relative positions of these nucleosides to favor the photoreaction. As shown in Fig. 4, No. 2, the presence of $Mg^{++}$and spermidine (1:3) does not further increase the rate of the photoreaction.

The photoproduct was generated maximally by prolonged irradiation in the presence of 0.001 M Mg^{++}, 0.1 M NaCl, 0.05 M Na cacodylate pH 7.0, and reduced by $NaBH_4$. The fluorescent tRNA was then dialysed against EDTA to remove Mg^{++}, resulting in a 50% decrease of fluorescence. This only partial loss of structure is a consequence of the covalent linkage generated by the photoreaction. As shown in Figure 4 - No. 3 a great excess of Mg^{++} was now required to increase the fluorescence intensity. However far smaller amounts of spermidine were fully active in this respect.

Several models have been proposed for the tertiary structure of tRNA. Srd is probably located in the groove of the arms containing dihydrouridine and CCA (11). Mg^{++} favors a geometry facilitating contact of Srd with the nonadjacent cytidine residue (9). The effects of spermidine 1) in partially enhancing the fluorescence of Srd, 2) in maximizing this activity in cooperation with Mg^{++}, 3) in most effectively facilitating the photoreaction, and 4) in reestablishing the appropriate geometry and fluorescence of the reduced photoproduct suggests that some molecules of spermidine are involved in the organization of three dimensional geometry of the limited helical region close to Srd. The shape of the structure in which spermidine fits is fairly specific since the various properties conferred by this base can not be matched by the natural bases, putrescine and spermine, or by Mg^{++} ion.

Acknowledgements: We wish to acknowledge the hospitality extended to Francois Pochon by Dr. Lloyd M. Kozloff and the excellent assistance of Miss L. Lapi.

References:

1. For general references see: Cohen, S. S. Introduction to the Polyamines., Prentice-Hall, Inc., Englewood Cliffs, N. J. (1971).
2. Hampel, A. and Bock, R. M., Biochemistry 9:1873 (1970).
3. Fresco, J. R., Adams, A., Ascinoe, R., Hemley, D., and Lindahl, T., Cold Spring Harbor Symp. Quant. Biol. 31:527 (1966).
4. Bluestein, H. G., Allende, C. C., Allende, J. E. and Cantoni, G. L., J. Biol. Chem. 243:4603 (1968).
5. Robison, B. and Zimmerman, T. P., J. Biol. Chem. 246:110 (1971).
6. Leboy, P. S., FEBS Letters 16:117 (1971).
7. Cohen, S. S., Morgan, S. and Streibel, E., Proc. Natl. Acad. Sci. 66:669 (1969).
8. Pochon, F., Balny, C., Scheit, K. H. and Michelson, A. M., Biochim. Biophys. Acta 228:49 (1971).
9. Favre, A., Michelson, A.M. and Yaniv, M., J. Mol. Biol. 58:367 (1971).
10. Leonard, N. J., Bergstrom, D. E. and Tolman, G. L., Biochem. Biophys. Res. Commun. 44:1524 (1971).
11. Labanauskas, M. F., Connors, P. G., Young, J. D., Bock, R. M., Anderegg, J. W. and Beeman, W. W. Science 166:1530 (1970).