THE BEHAVIOR OF 4-THIOURIDINE IN THE E. COLI s-RNA MOLECULE

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A new sulfur-containing nucleotide has been isolated as a normal constituent of the s-RNA of E. coli and identified chemically (1) as 4-thiouridylic acid (4-TUMP [1]). The ultraviolet absorption associated with this base appears as an additional absorption peak in the spectrum of native E. coli s-RNA, with a maximum at 336 mμ and an absorption 1.5-2% of that at 260 mμ (Fig. 1). Thus,

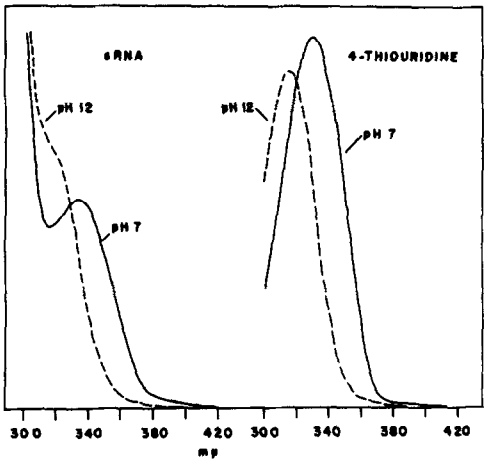

Fig. 1. Ultraviolet spectra of E. coli s-RNA and of 4-thiouridine. E. coli s-RNA was dissolved in 0.005 M phosphate, pH 7, or in 0.01 M KOH and read immediately. At pH 7, A_{260} is 20.8, A_{335} is 0.4. In the 4-TU curves, the same solvents were used. The concentration of 4-TU is 4.3×10^{-5} M; A_{331} at pH 7 is 0.74.

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[1] Abbreviations used are as follows: 4-TUMP, the 2'(3')-phosphate ester of 4-thiouridine; 4-TU, 4-thiouridine; poly U, polyuridylic acid; GMP, 2'(3')--guanosine monophosphate; DEAE-cellulose, diethylaminoethyl cellulose.

since in native s-RNA the absorption peak due to 4-TU is well separated from that of the bulk of the s-RNA nucleotides, it is possible to follow the behavior of 4-TU in intact s-RNA under varying conditions. The present report deals with the comparison between monomeric 4-TU and the s-RNA 4-TU with respect to pK and to denaturation with heat or urea.

MATERIALS AND METHODS

E. coli s-RNA was obtained from General Biochemicals, Chagrin Falls, Ohio. It was further purified by passage over a column of IRC-50, dialysis and lyophilization. Chemically synthesized 4-thiouridine disulfide was the gift of Dr. J. Fox, and another portion was obtained from Cyclo Chemical Co., Los Angeles, Calif. It was reduced to 4-thiouridine by treatment with a five-fold excess of 2-mercaptoethanol and purified by paper chromatography in 86% butanol-H_2O.

Spectrophotometric determinations were performed on a Zeiss spectrophotometer and in a Cary Model 14 recording spectrophotometer, each fitted with constant-temperature cell blocks connected to a circulating water bath.

RESULTS

It is apparent from the spectra in Fig. 1 that the thiopyrimidine in native s-RNA is capable of enolizing in alkaline solutions just as the free nucleotide does. A spectrophotometric determination of the pK of the free nucleoside and the s-RNA 4-TU was carried out by following the shift in absorption with pH. The results in Fig. 2 indicate that 4-TU has a much lower pK as the free nucleoside than when it is present as a constituent of s-RNA. The actual values are calculated from this titration to be 8.2 for the pK of 4-TU and 9.9 for the s-RNA 4-TU component. These values are unusually low, since the pK of uridine changes from 9.2 in the monomer to 10.1 for poly U (2), and GMP from 9.4 in the nucleotide to 11.2 in poly G (3). The magnitude of the change is consistent with the participation of the 4-TU moiety in the secondary structure of s-RNA, although this is not necessarily so from these data alone.

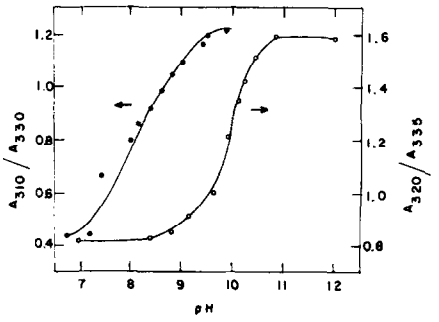

Fig. 2. Spectrophotometric titration of 4-TU and of the 4-TU component of s-RNA. Ratios of absorbancies at two wavelengths are used to decrease errors due to small differences in dilutions and to the difficulty of determining an exact absorption maximum in flat portions of a shifting curve. Open circles, s-RNA. One part of a solution of s-RNA in 0.5 M NaCl was diluted into four parts of 0.05 M Tris-Cl or glycine-NaOH buffers of the appropriate pH, and the absorbancies read at two wavelengths. The 4-TU curve (solid points) was determined in the same manner without the addition of NaCl. The optical densities at 330 mμ in the two sets of determinations were similar.

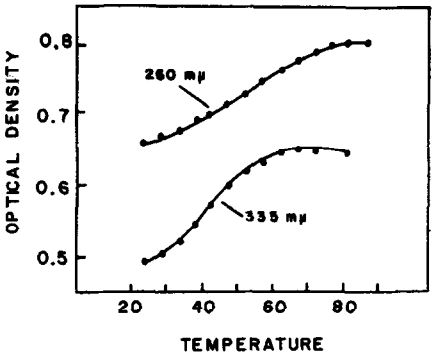

Fig. 3. Heat denaturation of native s-RNA at two wavelengths. The solvent was 0.005 M phosphate, pH 7. The curve at 335 mμ was obtained in a cell with a 1 mm light path. The curve at 260 mμ was obtained on the same solution diluted with four parts of buffer and read in a cell with a 1 cm. light path. Thus, the difference in concentration between the two solutions is only five-fold, although the actual difference in absorbancy at the two wavelengths is 50-fold.

Since thermal denaturation may also be used to destroy secondary structure, the effects of temperature were studied in native s-RNA. The 260 and 336 mμ absorption peaks were followed simultaneously as a solution of E. coli

s-RNA was heated in a thermostatically controlled cell block. Fig. 3 shows that the 4-TU peak, measured at 335 mμ, underwent a thermal transition similar to that observed at 260 mμ, but that the T_m for the 4-TU transition occurs about 10^o lower than that for the 260 mμ transition at this salt concentration. The percentage of hyperchromicity is approximately the same, 22%, at the two wavelengths after heating. The extent of the hyperchromicity at high temperatures is consistent with hydrogen-bonding of the 4-TU in the molecule. 2/

As has been shown by Felsenfeld and Sandeen (5), not all regions of the s-RNA molecule melt out at the same time. Regions rich in A-U base pairs are less stable than those containing a high proportion of G-C bonds. In the present instance, it is apparent that the 4-TU-containing region of s-RNA is melting out at a lower temperature. It must be remembered, however, that this is a whole population of s-RNA species, and that only about half of them, on a statistical basis, can contain even one 4-TU, as calculated from the percentage of 4-TU in whole s-RNA (1). We cannot say whether it is only the 4-TU-containing regions of s-RNA or the whole molecule of 4-TU-containing species which is denaturing at lower temperatures. Only measurements on a pure species of s-RNA containing the thiopyrimidine can provide this answer.

It has been noted that the absorption maximum in native s-RNA occurs at 336 mμ, while that of isolated 4-TU is seen at 331 mμ. When an alkaline hydrolysate of s-RNA is made and neutralized, the absorption maximum is found to shift to 331 mμ. No other constituents were present in the DEAE-cellulose column fractionation (1) which had sufficient absorption to account for the initial 336 mμ maximum. The question arose whether the alkaline hydrolysis had somehow changed the nature of the base, or whether it was truly present in the s-RNA as an unsubstituted thiopyrimidine. The thermal denaturation

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2/ Essentially the same results were obtained on heating in 0.003 M $MgCl_2$--0.005 M phosphate, pH 6.8 (4), except that the melting curves were much sharper and the difference in T_m between the two wavelengths was only about 3^o, i.e., 79 and 82.2^o at 335 and 260 mμ, respectively.

described above throws light on this question. It was found that the spectrum of the 4-TU region of the s-RNA, when measured at 70° where the structure is completely heat-denatured, now has an absorption maximum at 333 mμ, closer to

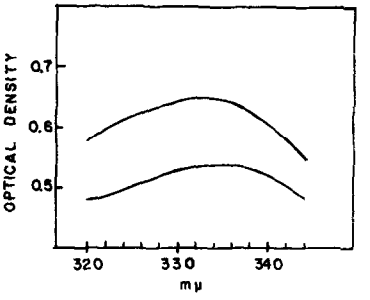

Fig. 4. Changes in spectrum with temperature. Ultraviolet spectra of the 4-TU region were obtained during the heat denaturation experiment of Fig. 3. Upper curve, 71.5°; lower curve, 24°.

that of the nucleotide, and that the 336 mμ peak reappears on cooling (Fig. 4). It seems quite probable that the abnormal maximum observed in undenatured s-RNA can be ascribed to the effects of the secondary structure in which the 4-TU participates rather than to any covalent alteration in the thiopyrimidine itself.

The effects of denaturation of the s-RNA in concentrated solutions of urea were also studied. Solutions of E. coli s-RNA were made in 0.005 M phosphate buffer, pH 6.8, and in the same buffer containing 8 M urea. Absorbancies were read without dilution at all wavelengths by employing cells of appropriate light paths. The hyperchromicities exhibited in the urea-treated s-RNA were 15.3 and 14.5% at 260 and 335 mμ, respectively. The spectrum in the 4-TU region was observed to shift from a maximum at 336 mμ in buffer to a maximum at 333 mμ in urea solution. This is the same type of shift as was seen on heat denaturation. The smaller amount of hyperchromicity observed with urea as compared to heat may indicate that the denaturation of the 4-TU bonding in s-RNA is not complete with urea.

On the basis of the relatively great shift in pK when 4-TU is bound into the s-RNA molecule and the spectral changes associated with denaturation of the molecule by heat or urea (i.e., the hyperchromicity and the shift in absorption maximum), it seems very likely that this thiopyrimidine is present in hydrogen-bonded form in s-RNA. [3/] The region of the molecule containing the 4-TU is relatively less stable than the bulk of the structure, as indicated by the 10° difference in T_m measured at 335 mμ. The actual bonding partner for 4-TU is s-RNA is unknown, although by analogy with uridine, it is likely to be adenosine at neutral pH.

SUMMARY

A study has been made of the state of the sulfur-containing nucleotide, 4-thiouridine, in the s-RNA from E. coli. The evidence gathered from titration and denaturation studies indicates that this base is present in hydrogen-bonded form in normal E. coli s-RNA.

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3/ Evidence that disulfides can be produced in s-RNA by iodine treatment has recently been presented by Carbon et al. (6), along with the description of an isomer of 4-TU, namely, 2-thiouridine or a closely related base. Iodine treatment of s-RNA produces changes in the 4-TU region of the s-RNA spectrum consistent with such disulfide bonds, and a study of the physical properties of such s-RNA will be the subject of a further paper.