THE PHOTOCHEMISTRY OF 4-THIOURIDINE IN ESCHERICHIA COLI t-RNA$_I^{Val}$.

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The following note describes a specific modification of t-RNA$_I^{Val}$. Irradiation at 335 mμ of t-RNA in aqueous solution causes a local change in the conformation of the molecule, the result of a specific quantitative modification of the 4-thiouridine. This photoreaction occurs only when the 4-thiouridine in the 8th position interacts strongly with a non-adjacent base : a cytosine residue in 13th position.

Previous studies on the modification of t-RNA by UV irradiation, in the range 260 to 280 mμ (Harriman and Zachau, 1966, Schulman and Chambers, 1968) or by photodynamic reaction (Kuwano et al, 1968) are somewhat difficult to interpret since numerous residues in the molecule were modified. The 4-thiouridine (4 TU) residue present in certain t-RNA of E. coli (Lipsett, 1966) has an absorption band in the region of 330 mμ and hence can be modified specifically by irradiation at this wavelength.

Recently Pleiss et al (1969) reported that irradiation at 330 mμ of total E. coli t-RNA in tert-butanol converted the thiouridine into uridine (or cytidine in the presence of ammonia). Under such conditions the RNA is none-structured. A different reaction occurs in aqueous solutions under conditions where secondary structure is maintained and we describe the effect of irradiation at 335 mμ of pure t-RNA$_I^{Val}$.

MATERIALS AND METHODS

Valine specific t-RNA, prepared by fractionation on benzoylated DEAE-cellulose columns (Gillam et al, 1968) as described by Yaniv and Barrell

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(1969) from total E.coli t-RNA, or from fractions enriched in t-RNA^{Val} by counter current distribution, was irradiated in cacodylate buffer (pH 7) at ordinary temperature with monochromatic light at 335 mμ (band width 10 mμ).

The UV absorption spectrum showed characteristic changes in the region of absorption of 4-thiouridine during the course of irradiation (Fig. 1). Two isobestic points appear, suggesting direct conversion of 4-TU to a single new product. The reaction was followed either by the decrease of the fluorescence of the 4-TU in the RNA (unpublished results) or by variations in absorption at 320 or 360 mμ ; at least 90 % of the 4-TU was transformed with pseudo-first order kinetics (Fig. 3). The quantum yield as determined after calibration of the monochromator with a ferrioxalate actinometer (Hatchard and Parker, 1956) was of the order of 5×10^{-3} mole/E.

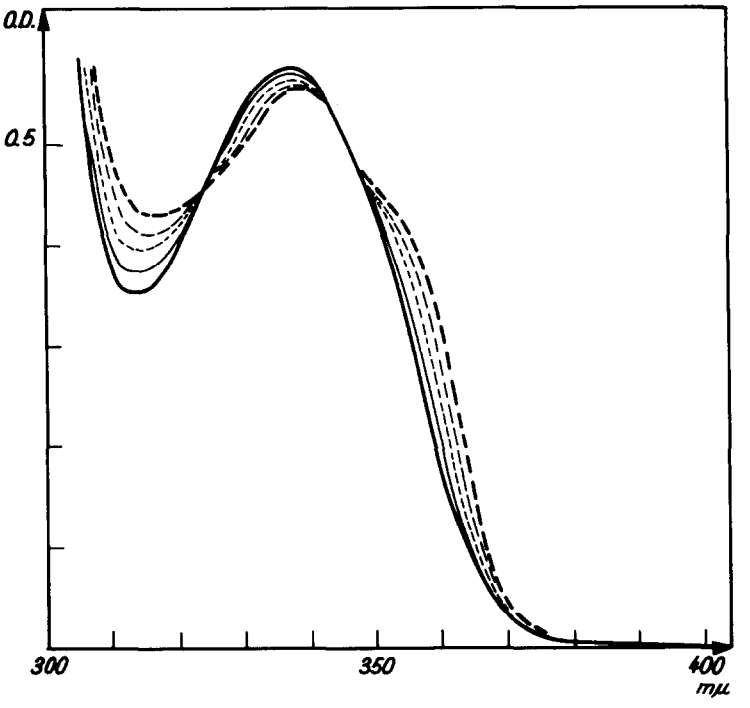

Figure 1 : Variation of t-RNA$_I^{Val}$ absorption during irradiation.
The absorption in the 335 mμ region is specific for the 4-TU residue. t-RNA was irradiated in 0.01 M cacodylate buffer pH 7.0 containing 0.15 M NaCl with a Bausch and Lomb monochromator at 335 mμ. UV absorption was measured with a Cary 14 spectrophotometer :
initial absorption —, after 0.5 hrs —, ... 1 hr, ----- 4 hrs, – – – 8 hrs of irradiation.

RESULTS AND DISCUSSION

Although large changes occur in spectroscopic properties (circular

dichroïsm, temperature-ultraviolet absorption at 330 mμ profiles) in the region of absorption specific to thiouridine, only small variations could be detected in the range 200 to 300 mμ. X-ray scattering by normal and irradiated t-RNA were very similar (Ninio, 1969). It thus appears that modification by irradiation of the thiouridine causes only a local change in conformation rather than a major alteration of the entire molecule of t-RNA. It may be emphasised that irradiation of thiouridine or of endonuclease digests of t-RNA^{Val} do not give rise to the same modification for which the integrity of secondary structure of the t-RNA is necessary.

Further information on the nature of the reaction induced by irradiation of t-RNA^{Val} at 335 mμ was obtained by spectrophotometric and fluorescence titration studies of the thiouridine in native t-RNA (Fig. 2). Two pK values of 4.3 and 9.6 were observed. That of 9.6 corresponds to ionisation of thiouridine by loss of a proton, with the usual displacement to higher values in a

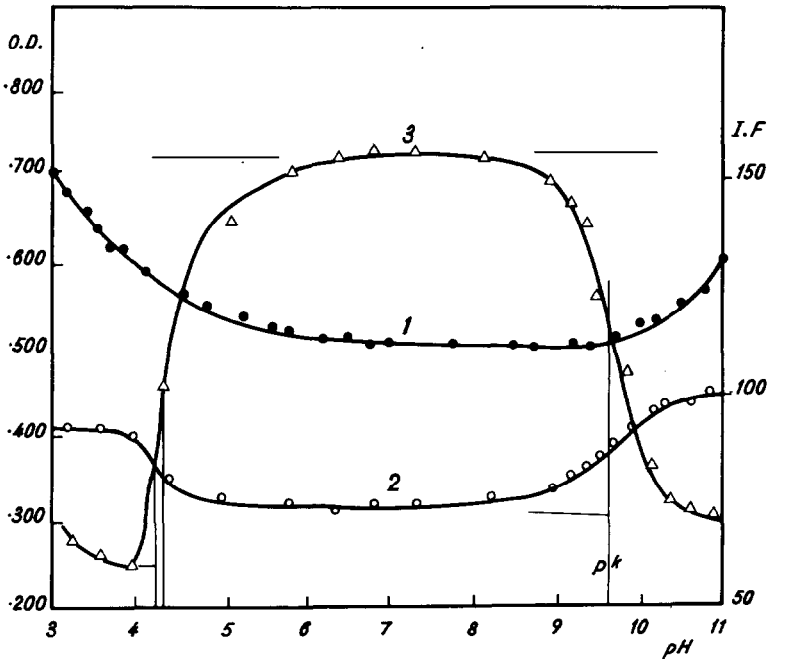

Figure 2 : Spectrophotometric titration of native t-RNA$_I^{Val}$ in 0.15 M Na^+ at 20° C.
1) absorption measurements at 280 mμ
2) absorption measurements at 330 mμ
3) fluorescence of 4-TU (λ ex 350 - λ em 510).

structured polymer compared with the pK of 8.1 for the monomer (Lipsett, 1966). The lower pK is not present in the monomer and the semi-cooperative nature of the transition tends to exclude an indirect effect of a large number of bases.

Previous studies (Michelson and Pochon, 1969) suggest that this pK corresponds to a protonation of a cytosine residue (either single strand or a G-C base pair) in direct interaction with the thiouridine.

Formation of the specific photoproduct in t-RNA can be readily followed by subsequent reduction with sodium borohydride to a compound with new fluorescent characteristics. No such product was observed when the RNA was irradiated at a pH lower than 3.8 (protonation of cytosine residues) although quantitative yields are obtained in the range of pH 5 to 9. Since the primary sequence of t-RNA_I^{Val} does not contain a cytosine residue next to the 4-thiouridine (Yaniv and Barrell, 1969), interaction with a non-adjacent base is necessary for the reaction to occur. Sequence analysis of irradiated t-RNA_I^{Val} (Yaniv et al, 1969) shows formation of a covalent bond between 4-TU in the 8th

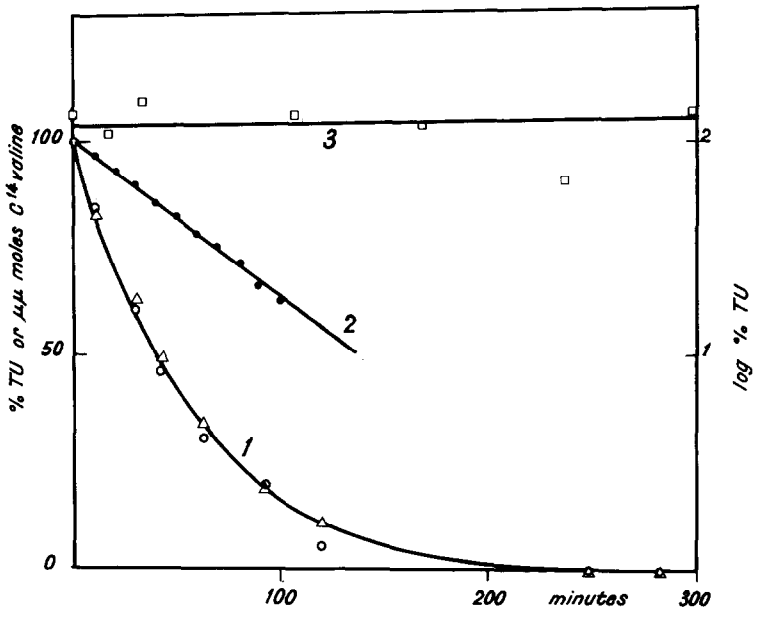

Figure 3: Kinetics of photoreaction of 4-thiouridine (4-TU) in t-RNA_I^{Val} ($0.3\ OD_{330}$) irradiated at 335 mμ and in cacodylate buffer pH 7.0, 0.15 M Na^+ at 4°C. Curve 1 (000) represents the percentage (x) of intact 4-TU at time t

$$x = \frac{Dt - D_o}{Df - D_o}$$

calculated from increase in optical density at 360 mμ where D is the optical density at zero time (D_o) and final (Df)

or $$x = \frac{-F_o - F}{F_o - Ff}$$

from decrease in fluorescence (Δ Δ Δ) where F is the fluorescence of 4-thiouridine (λ ex 350 - λ em 510).
Curve 2 shows the first-order kinetics. Curve 3 represents the charging activity of the t-RNA as a function of irradiation time measured with an excess of pure valyl-t-RNA synthetase.

position and cytosine in the 13th position. The relatively small changes in UV absorption suggest that the photochemically induced reaction involves N3, C5 or C6 of the pyrimidine ring of thiouridine rather than known reactions such as hydration, dimerisation or displacement of sulphur.

The irradiated t-RNA is fully amino acylated (Fig. 3), but the affinity for valine t-RNA synthetase shows a three fold decrease. Affinity for the ribosome poly UG complex remains unchanged (Yaniv and Favre, 1969). It is clear that the above results provide a stereochemical restriction for the tertiary structure of t-RNA$_I^{Val}$ since close proximity of thiouridine (position 8) and cytidine (position 13) must be accomodated. A possible model will be described elsewhere (Ninio et al, 1969). Interaction between thiouridine and the cytosine residue of the fourth base pair of the dihydrouracil stem is also indicated for other t-RNA from E. coli. Thus t-RNA$_2^{Val}$, t-RNA$_M^{Met}$, t-RNA$_F^{Met}$, t-RNA^{Phe} gave the same characteristic product on reduction with sodium borohydride after irradiation of the RNA (unpublished results). Presence of thiouridine at position 8 and cytidine in position 13 has been indicated for these RNA (Yaniv and Barrell, unpublished, Cory et al, 1968, Dube et al, 1968, Barrell and Sanger, 1969). In the case of t-RNA$_2^{Tyr}$ which lacks a G-C base pair at this location (Goodman et al, 1968), no such product was obtained, again indicating a high structural specificity for the reaction. Thus a common tertiary structure in the region of thiouridine is indicated for at least five t-RNA species and is not excluded for others.

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