PHOTOCHEMICALLY INDUCED TRANSITION OF 4-THIOURIDINE TO URIDINE OR URIDINE AND CYTIDINE IN E. COLI TRANSFER RIBONUCLEIC ACID.

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Little is known about the contribution of the minor nucleotides to the functional properties of tRNA. Although impressive progress has been made for the chemical synthesis of the gene for alanine tRNA from yeast (Kumar et al., 1968) the total synthesis of a tRNA containing the minor bases is not expected in the near future. The selective chemical modification of specific minor components in tRNA provides an approach for the study of the functional role of the minor nucleotides avoiding de novo synthesis of tRNA. Modification of a minor but not of a major component allows the alteration of topographically well-defined regions for tRNA species with known primary structure.

A novel reaction is described in this paper which selectively transforms the minor nucleoside 4-thiouridine to uridine or uridine and cytidine in E. coli tRNA under extremely mild photochemical conditions.

Results

Irradiation of 4-thiouridine in air-saturated tert. butanol with a mercury high pressure lamp filtered by a Pyrex filter or with monochromatic light at 330 mμ leads to the rapid and virtually quantitative formation of uridine. Isosbestic points were observed in the absorption spectrum measured as a function of light dose. No reaction is observed in the absence of air

or oxygen. The pseudo-first order rate constants for the disappearance of 4-thiouridine and the formation of uridine were 0.10 min^{-1} and 0.08 min^{-1}, respectively (Figure 1). No significant change in the rates was found when a constant stream of air was passed through the photolysis solution. Irradiation of 4-thiouridine in water on the other hand leads to a complex mixture of products resulting, in part, from addition of water to the 5,6-double bond (Ochiai and Cerutti, unpublished results).

Cytidine and uridine were obtained in a ratio of approximately 1 : 1 when a mixture of ammonia and air (1 : 2) was passed through the photolysis

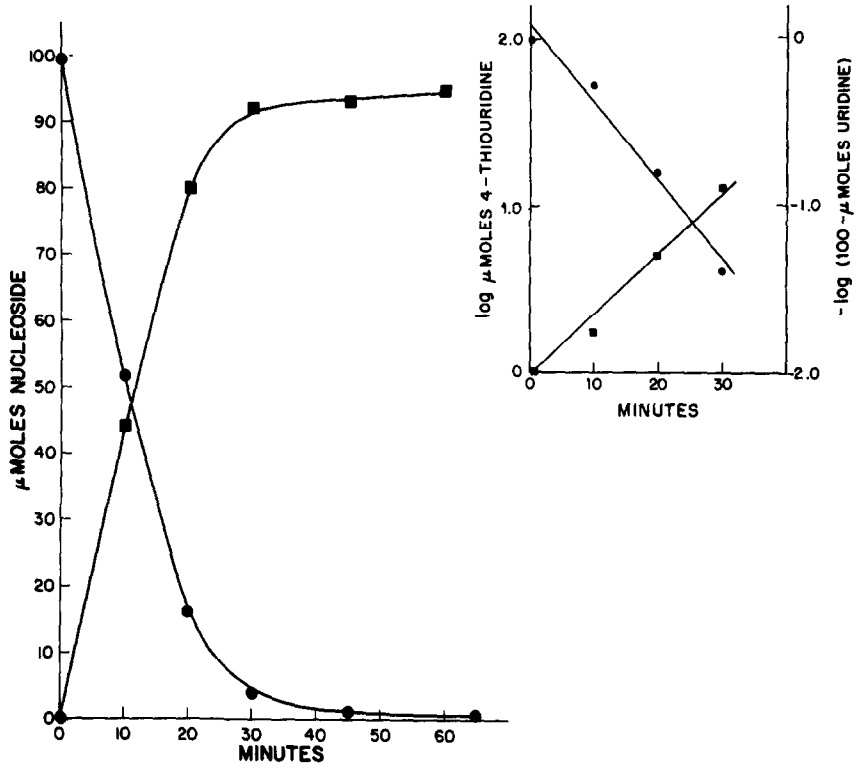

Fig. 1 - Phototransformation of 4-thiouridine to uridine in tert butanol.
●—●, disappearance of 4-thiouridine calculated from the absorption at 330 mμ in 0.01M phosphate buffer pH 7 ($\varepsilon = 21.2 \times 10^3$).
■—■, formation of uridine calculated from the absorption at 262 mμ in 0.01M phosphate buffer pH 7 ($\varepsilon = 10 \times 10^3$).
Irradiation with a 200 watt mercury high pressure lamp with a Pyrex filter sleeve in air-saturated tert. butanol.
Inset: semi-log plot of the same data demonstrating pseudo-first order kinetics.

solution. Variation of the composition of the gas mixture influenced the ratio of the two products substantially; cytidine and uridine were obtained in an approximate ratio of 2 : 1 when a mixture of ammonia and air (1 : 1) was passed through the solution. However, some uridine was generated even when 4-thiouridine was photolyzed at -78° in liquid ammonia. The products were identified by thin-layer chromatography and separated on DEAE-paper and Dowex 50W-X8(H^+) (*see also* legend to Figure 2).

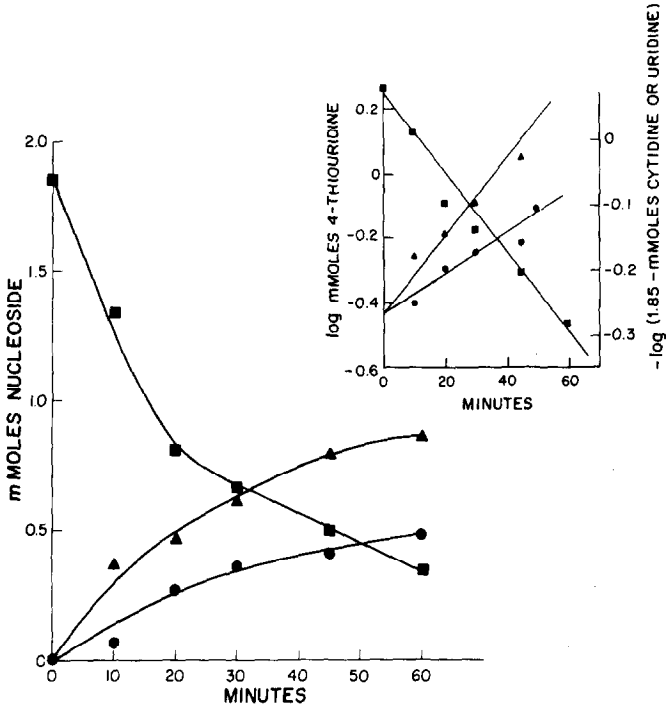

Fig. 2 - Phototransformation of 4-thiouridine to uridine and cytidine in tert. butanol.
■—■, disappearance of 4-thiouridine calculated from the absorption at 330 mμ in 0.04M phosphate buffer pH 7.
▲—▲, formation of cytidine calculated from the absorption at 279 mμ in 1N HCl ($\varepsilon = 13.4 \times 10^3$).
●—●, formation of uridine calculated from the absorption at 262 mμ in 0.04M phosphate buffer pH 7 ($\varepsilon = 10 \times 10^3$); a correction was made for the absorption of 4-thiouridine at 262 mμ.
Irradiation conditions see legend to Figure 1. A 1 : 1 mixture of dry air and ammonia (40 ml/min) was passed through the photolysis solution. Gas flow and the composition of the gas mixture was monitored by flow meters. Cytidine was removed from the samples by passage through Dowex 50W-X8(H^+) for the determination of uridine and 4-thiouridine. Cytidine was obtained by elution with N sodium hydroxide.
Inset: semi-log plot of the same data demonstrating pseudo-first order kinetics.

The reaction was highly selective for 4-thiouridine. The major nucleosides and the minor nucleosides pseudouridine, 1-methyladenosine, glucosyl-2-thiouracil (used as a model compound for the corresponding riboside) and N^6-isopentenyladenosine were photochemically inert when irradiated at longer than 300 mμ in tert. butanol as judged by absorption spectroscopy and thin-layer chromatography. The hexadecyltrimethylammonium salt of E. coli tRNA, prepared according to Weil and Ebel (1962), was irradiated with monochromatic light at 330 mμ in tert. butanol in air or in an ammonia-air atmosphere. The dose dependent loss of the absorption at 335 mμ originating from the 4-thiouridine residues in the tRNA salt was found to follow pseudo-first order kinetics with a rate constant of 9×10^{-3} min^{-1} at ambient temperature in air-saturated tert. butanol. The rate constant

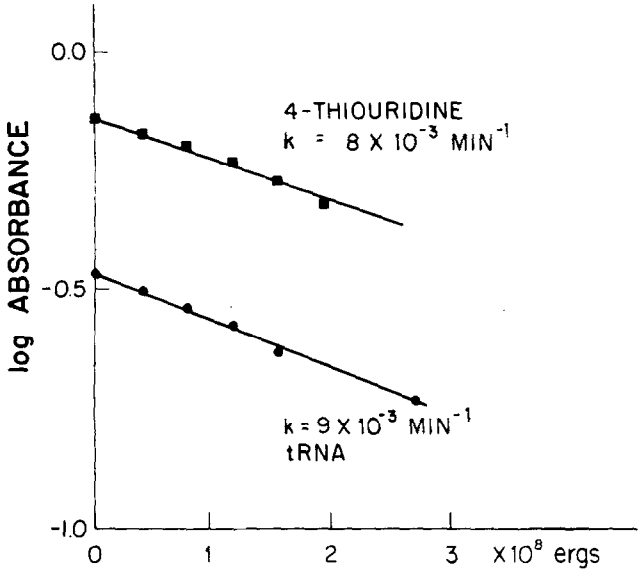

Fig. 3 - Kinetics of the disappearance of free 4-thiouridine and 4-thiouridine in E. coli tRNA (hexadecyltrimethylammonium salt).
■—■, disappearance of free 4-thiouridine; log of the absorbance at 335 mμ in tert. butanol.
●—●, disappearance of 4-thiouridine in E. coli tRNA (hexadecyltrimethylammonium salt); log of the absorbance at 335 mμ in tert. butanol.
Irradiation with monochromatic light at 330 mμ using a 1200 line Bausch & Lomb grating monochromator (2500 watt Xenon high pressure lamp). Quantum flux determined with a thermopile and a ferrioxalate actinometer (Hatchard and Parker, 1956).

for the photoreaction of the monomer under the same conditions was a comparable 8×10^{-3} min^{-1} (Figure 3). A pseudo-first order rate constant of 6×10^{-3} min^{-1} for the photoconversion of 4-thiouridine in the tRNA salt was obtained when a stream of a mixture of ammonia and air (1 : 1) was passed through the photolysis solution. No fragmentation of the tRNA subsequent to exposure to irradiation in an ammonia - air atmosphere could be detected from the elution pattern of the irradiated sample on Sephadex G100 (10^{-3}M tris-buffer, pH 7).

Discussion

The novel reaction described in this paper involves the mild photooxidation of 4-thiouridine to an intermediate which readily undergoes hydrolysis or ammonolysis to uridine or cytidine, respectively. The intermediate may well be N-ribosyl-2-oxy-pyrimidine-4-sulfonate or the corresponding sulfinate. There are several examples in the literature, which support this interpretation. The oxidation of thiopyrimidines to the corresponding sulfinates and sulfonates and the hydrolysis of these intermediates to the corresponding pyrimidinones has been described (Brown, 1962; Taylor and Cheng, 1960). Most significantly N-(2'-deoxyribosyl)-2-oxy-pyrimidine-4-sulfonate has been demonstrated (Ziff and Fresco, 1968) as an intermediate in the oxidation of 2'-deoxy-4-thiouridine with sodium periodate (cf. Lipsett, 1965). No direct evidence for the formation of a sulfonate intermediate has been obtained in our experiments. However, sulfite ions were detected in the photolysis solution thus supporting the notion that a sulfonate intermediate is formed. The mild photooxidative conditions, in contrast to the much more vigorous conditions of the oxidation with periodate, may lead to a relatively low stationary concentration of the intermediate. Low levels of the sulfonate or sulfinate in the presence of relatively large amounts of starting material or product may well escape detection by the spectrophotometric techniques used in our experiments.

The presence of 4-thiouridine has been detected in phenylalanine, valine,

tyrosine and tyrosine su^+ tRNA from E. coli ($tRNA_{phe}$, Uziel, 1966; $tRNA_{tyr}$, Lipsett and Doctor, 1967; $tRNA_{tyrsu^+}$, Goodman et al., 1968). The number and localization of the 4-thiouridine residues in these polymers is not yet known with the possible exception of tyrosine tRNA. Detection and localization of 4-thiouridine in oligonucleotides in the sequence elucidation of E. coli tRNA is hampered by the instability of 4-thiouridine towards mild acid and base (cf. Madison, 1968). A recently developed method for the selective reduction and labelling of 4-thiouridine in tRNA with sodium borotritiide (Cerutti et al, 1968) circumvents this difficulty. The selective photochemical conversion of 4-thiouridine in the presence of $[C^{14}]$methylamine into the stable derivative N^4-$[^{14}C]$methylcytidine offers an alternative solution to this problem.

The biosynthesis of 4-thiouridine in E. coli tRNA involves the thiolation of specific uridine residues in the preformed polymer (Lipsett et al., 1967). The modified tRNA obtained by the selective photochemical transformation of 4-thiouridine to uridine may therefore be considered a precursor in the biosynthesis of tRNA.

Studies of the effect of the iodine oxidation of the thiopyrimidine nucleotides in E. coli tRNA (Carbon et al., 1965; Lipsett, 1966) and in B. subtilis tRNA (Doi & Gohler, 1966) did not yield conclusive information about the functional role of these minor components especially since the influence of the modification on the secondary and tertiary structure of the tRNA cannot be assessed. The phototransformation of 4-thiouridine to uridine and cytidine induces the transition of a minor to a major nucleoside in tRNA. No unnatural nucleoside derivative with unpredictable effects on the properties of the tRNA is formed in this reaction. The ribose portion of the 3'-terminal adenosine residue of tRNA is not affected by the mild and highly selective oxidation conditions used in these experiments. Part of the usual difficulties in the interpretation of the effects of chemical modifications on the biological activity of tRNA are therefore avoided in this case.

It is hoped that a comparison of the biological activity of tRNA preparations containing at identical locations in their primary structure either 4-thiouridine, uridine or cytidine will yield new insight into the functional role of 4-thiouridine.

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