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THE REACTION OF 4-THIOURIDINE WITH CYANOGEN BROMIDE

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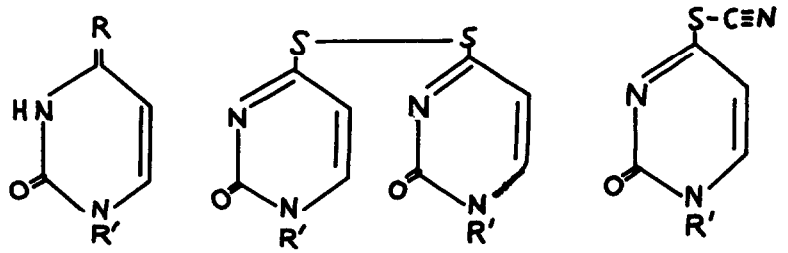
Elsewhere[1] we have reported preliminary results on the reaction of cyanogen bromide (BrCN) with 4-thiouridine(I) and the 4-thiouridine residues in two pure tRNAs from Escherichia coli. The reaction when 4-thiouridine was heated in phosphate buffer at 100° for 3 min. with an excess of BrCN lead to the quantitative production of uridine. We would here like to present a reaction pathway for this reaction. Other workers have reported on the reaction between 4-thiouridine derivatives[2] and BrCN under different conditions and the results presented here differ significantly from theirs.



R′= ribosyl

I R=S II III

IV R=O

The addition of 1.0 mole of 4-thiouridine to 0.5 mole of BrCN resulted (at pH 6.5 in 0.03M phosphate buffer) in the immediate disapperance of 4-thiouridine from the solution. The product was identified by its mobility on TLC silica plates in several solvent systems and by its UV spectrum in ethanol (λ max 320nm, ε 29,750; λ max 260nm, ε 6,500; λ min 280 nm, ε 5400) as the

disulphide(II). 4-Thiouridine could be quantitatively recovered by the addition of 2-mercaptoethanol. With the addition of more BrCN the disulphide further reacted at pH 6.5 if the solution was heated to 37° for 30 min to give a mixture of 4-thiouridine and a compound X. At pH 8.5 in phosphate buffer the further reaction of the disulphide is much quicker and the following results were obtained with the addition of differing amounts of BrCN, (the figures give the percentage of the original 4-thiouridine converted into each compound from a knowledge of the UV absorption spectra and their extinction coefficients, the amount of X being determined by difference).

Moles of BrCN added per mole of 4-thiouridine	Time of incubation at 37°(min)	% 4-thiouridine converted into:		
		4-thiouridine	disulphide	X
0.3	< 1	51	32	17
	30	65	9	26
0.9	< 1	0	46	54
	30	8	0	92
1.2	< 1	0	49	51
	30	0	0	100

The amounts of compound produced at any time as given above can be accommodated in the overall scheme:

2 moles 4-thiouridine $\xrightarrow{1 \text{ mole } Br^+}$ 1 mole 4-thiouridine disulphide $\xrightarrow{1 \text{ mole } CN^-}$ 1 mole 4-thiouridine + 1 mole X. This leads to an overall reaction of the type as previously identified:

4-thiouridine + BrCN ⟶ X (4-thiocyanatouridine, III). Confirmation that the reacting species in the first part of the reaction was Br^+ was obtained from the reaction of 4-thiouridine with N-bromsuccinimide which resulted in the production of 4-thiouridine disulphide. Furthermore the second part of the reaction, that of a disulphide with cyanide ion, is a well-known reaction. This reaction was studied further as follows:

4-Thiouridine disulphide in 0.03M phosphate buffer pH 8.5 was treated with differing amounts of potassium cyanide as shown below.

Moles of CN^- added per mole of disulphide	Time of incubation at 37° (min)	% 4-thiouridine present as:		
		4-thiouridine	disulphide	X
0.5	< 1	11	78	11
	30	25	50	25
1.0	< 1	19.5	61	19.5
	30	50	0	50
3.0	< 1	38	24	38
	30	50	0	50

The amounts of compound produced at any time as given above can be accommodated in the overall scheme:

$$\text{1 mole 4-thiouridine disulphide} \xrightarrow{\text{1 mole } CN^-} \text{1 mole 4-thiouridine + 1 mole X.}$$

The fact that the compound (X) formed from the reaction of KCN on the disulphide and that formed from the reaction of BrCN on 4-thiouridine were identical, was demonstrated by their having identical R_f values in several solvent systems on TLC. They also had identical UV spectra in ethanol (λ max 310 nm, ε 8,000; λ max 250 nm, ε 7,800; λ min 280 nm, ε 4,000) and identical IR spectra (KBr disc) over the range 700 - 4000 cm^{-1} with a very sharp band at 2175 cm^{-1} (S-C$\equiv$N). A high resolution mass spectrum of X from a TLC plate eluate showed a molecular ion peak at 285.0422, corresponding to the formula $C_{10}H_{11}N_3O_5S$ (calculated 285.0419). It thus seems probable that X is 4-thiocyanatouridine III.

The compound X as isolated by TLC was heated in 0.05M phosphate buffer pH 6.5 and 8.5 at 100° and the results are given below.

Time of heating at 100° (min)	pH	% compound present in solution		
		4-thiouridine	uridine	X
0	6.5	0	0	100
	8.5	0	0	100
3	6.5	12	30	58
	8.5	30	70	0
5	6.5	18	48	34
10	6.5	25	66	9
20	6.5	26	72	2
	8.5	30	70	0

When heated in the presence of an excess of BrCN at pH 8.5 for 3 min at 100° compound X was quantitatively converted into uridine (IV).

Thus it has been shown that the initial reaction of BrCN with 4-thiouridine, in the presence of phosphate buffer at pH 6.5 and 8.5, gives the disulphide and not the thiocyanate which has a UV spectrum similar to, but easily distinguishable from, the disulphide. The disulphide reacts slowly at pH 6.5 and more rapidly at 8.5 to give a compound which has the properties expected of a thiocyanate. The thiocyanate is relatively stable at pH 6.5 but at pH 8.5 it decomposes at 100° in 3 min to give a mixture of uridine and 4-thiouridine in the ratio of 7:3. It has not been possible to produce only uridine from the thiocyanate except in the presence of an excess of cyanogen bromide to recycle the 4-thiouridine produced.

The phosphate buffer is essential for the reaction; in other buffers tried the thiocyanate was usually more stable and products other than uridine were formed. In fact if ammonium bicarbonate is used, cytidine is produced together with some uridine and other unidentified compounds. In a tRNA such as $tRNA_{II}^{Tyr}$ from Escherichia coli which has two adjacent 4-thiouridine molecules, spectrophotometric evidence was obtained that again the initial product of the reaction with BrCN was the intramolecular disulphide. However in the case of $tRNA_f^{Met}$ and for $tRNA_{II}^{Tyr}$ once the one 4-thiouridine molecule has been converted to uridine, it is unlikely that intermolecular disulphide bonds will form. In this case the initial thiobromide formed must be attacked by the nucleophile CN^- to give the thiocyanate directly rather than by another molecule of 4-thiouridine which under the conditions of these experiments is obviously the better nucleophile.

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