STABILITY OF 4,4'-DI(THIOURIDINE) AND 4-THIOURIDINE IN ALKALI*

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Summary: Cation exchange chromatographic analysis can distinguish between the alkaline hydrolysis products of 4-thiouridine and 4,4'-di(thiouridine). Although 4-thiouridine is the primary stable alkaline hydrolysis product from either of the above compounds, the disulfide [4,4'-di(thiouridine)] yields a second, unidentified compound. This latter substance is less stable at 80° than at 37°.

The discovery that sulfur-containing bases are constituents of transfer RNA (Carbon et al., 1965; Lipsett, 1965a; Schleich and Goldstein, 1965) raises the question as to their behavior in the conventional chemical hydrolytic procedures for polyribonucleotide analysis.

Both Schleich and Goldstein (1965) and Lipsett (1965a) obtain 50% recoveries of sulfur when alkaline hydrolysates of tRNA are chromatographed on Dowex-1. In both the above cases, the hydrolysis was carried out in 0.3 N NaOH for 18 hours at 37°. On the other hand, this low recovery[1,2] was not encountered with a highly purified $tRNA^{Phe}$. Analysis of the spectrum of the intact $tRNA^{Phe}$ and analysis after alkaline hydrolysis[3] both gave ca. 0.8 residue of 4-Srd per mole of $tRNA^{Phe}$. The difference in recoveries between crude tRNA (Lipsett, 1965b; Schleich and Goldstein, 1965) and a purified tRNA reflects the complexity of the starting material as well as the complexity of the hydrolysis mechanism(s).[4]

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[1] Abbreviations: 4-Srd = 4-thiouridine; 4,4'-$(Srd)_2$ = 4,4'-di(thiouridine); Urd = uridine (see IBG #7, Memo #399).

[2] D. Kelmers, to be published.

[3] M. Uziel, unpublished results.

[4] Cleavage may occur at either the −S−S− or the C−S bond. In the former case, 4-Srd and its sulfenic acid are formed initially; in the latter case, uridine and its disulfide should be formed initially (Reid, 1960).

The hydrolysis products of 4-thiouridine and 4,4'-di(thiouridine) and their relation to the parent molecule are the subject of this communication.

4,4'-$(Srd)_2$ was purchased from Cyclo Chemical Corp. This material contained 6.5% 4-Srd and 6% Urd. 4-Srd was prepared from 4,4'-$(Srd)_2$ by reduction with $Na_2S_2O_3$ (Carbon et al., 1965). The excess reagents and by-products were removed by gel filtration through Bio-Gel P-2. Hydrolyses were performed in micro polyethylene test tubes (Beckman Cat. #314326). A stock solution of 4,4'-$(Srd)_2$ in water (0.5 mg/ml) was prepared by dissolving the suspension at 37°. The following molar extinction coefficients[5] at 260 mμ were determined with this stock solution: 4,4'-$(Srd)_2$, $\epsilon = 11,200$; 4-Srd, $\epsilon = 3350$; X, $\epsilon = 7,400$.[6] Uridine has a molar extinction coefficient of 9900.

A rapid-ion-exchange microanalytical system[7] was employed for the analysis, using a recording spectrophotometer equipped with a wavelength-changing device (Anderson et al., 1963). 4-Srd is quantitatively recovered from the column, whereas only 80% of the 4,4'-$(Srd)_2$ is recovered.

Aliquots of both 4-Srd and 4,4'-$(Srd)_2$ were adjusted to 0.3 $\underline{N}$ NaOH and subjected to hydrolysis at 37° for 18 hours or at 80° for varying periods of time. The samples were brought to pH 4.7 with acetic acid and analyzed. Table I shows analytical values for the time course of the hydrolysis of 4,4'-$(Srd)_2$ and 4-Srd at 80°, and Fig. 1 shows the hydrolysis of 4,4'-$(Srd)_2$ at 37°.

Of the four compounds appearing in the column effluent, only 4-Srd and Urd are stable to the alkaline treatment. 4,4'-$(Srd)_2$ breaks down to Urd (10%), Srd (50%), and X. The same amount of Urd is formed either at 37° or at 80° (Table I), which suggests it is formed by a process independent of the one that forms X.

The latter compound is observed during hydrolysis and with a yield that is higher at 37° than at 80°. The absorbancy due to X in the analysis disappears after 40 minutes at 80°. The loss of the absorbing group could occur by ring-opening (Cohn and Doherty, 1956). In addition, $Na_2S_2O_3$ (Carbon et al., 1965) causes the complete transformation of X into 4-Srd.

Depending upon the mechanism of cleavage of 4,4'-$(Srd)_2$, either of two pairs of ultraviolet-absorbing compounds should be formed initially. Since both Srd and Urd

[5] All extinction coefficients were measured at pH 4.7.

[6] See Table I, footnote b.

[7] W. E. Cohn and M. Uziel, Abstract for the 150th American Chemical Society Meeting, September 1965.

TABLE I — Alkaline hydrolysis of thiouridine[a]

(all values in nanomoles)

Time	Temp (°C)	4,4'-(Srd)$_2$				Srd			
		4,4'-(Srd)$_2$	4-Srd	X[b]	Urd	4,4'-(Srd)$_2$	4-Srd	X	Urd
0	80	56				2	161	0	-
20 min	80	tr	45	10	8	0	145	0	4
40 min	80	0	57	3	8	0	153	0	9
60 min	80	0	57	0	11	0	138	0	9
18 hr[c]	37	0	38	53	9				

[a]Corrections have not been made for the 20% loss of 4,4'-(Srd)$_2$ during analysis. Corrections have been made for the Urd and 4-Srd initially present. 4-Srd and 4,4'-(Srd)$_2$ were hydrolyzed at 80° in 0.3 N NaOH. Separate tubes initially containing 0.67 absorbance units (260 mμ) in 0.1 ml were neutralized with acetic acid at 20, 40, and 50 minutes. The zero-time sample was made 0.3 N in acetic acid and immediately neutralized with NaOH. The total sample was then applied to a 28 cm X 0.5 cm^2 column of CG-120 (type III),[7] and the column was developed with 0.4 M NH_4 formate pH 4.7 at 50°.

[b]The values for compound X are, of course, only a first approximation, and the calculation of the molar extinction coefficient is based upon a conversion of 4,4'-(Srd)$_2$ into equimolar amounts of 4-Srd and X.

[c]See legend to Fig. 1.

are formed, both mechanisms may be operating, but in 0.3 N NaOH the −S−S− cleavage to 4-Srd and its sulfenic acid predominates.

Although these data were obtained with the nucleosides, there is little reason to expect alkaline hydrolysis of the nucleotides to differ qualitatively. Thus, the recovery of 4-Srd from alkaline hydrolysates does not unequivocally prove its presence in the parent molecule, although the isolation of compound X suggests the presence of a disulfide form of 4-thiouridine.

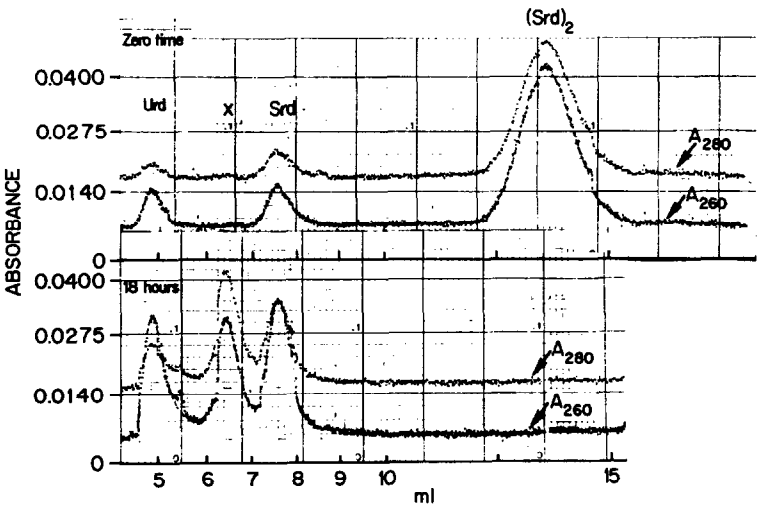

Fig. 1. 4,4'-$(Srd)_2$ was hydrolyzed in 0.3 N NaOH at 37° for 18 hours and then neutralized with acetic acid. The zero-time sample was not exposed to either acid or alkali. A 25-µl sample was analyzed on a 29 cm X 0.28 cm^2 column of Cg 120[7] with a flow cell having a 3 cm light path. The flow rate was 0.136 ml/min. The material labeled X appears more stable to hydrolysis at 37° than at 80°.

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