# CHEMICAL TRANSFORMATION OF 4-THIOURIDINE WITH NITROUS ACID

Shigeru Iida and Hikoya Hayatsu

Faculty of Pharmaceutical Sciences,

University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

**Received February 22, 1971**

Summary. Nitrous acid brought about the transformation, 4-thiouridine ⟶ bis(4-thiouridine) disulfide ⟶ uridine. The progress of the reaction was followed spectrophotometrically, and the products were identified by a combination of paper chromatography and UV spectra. The reaction was faster at pH 4.0 than at pH 4.9. At both pHs, this transformation occurred much more rapidly than the deamination, by nitrous acid, of guanosine, adenosine or cytidine. This finding provides a note of caution for those using nitrous acid as a tool to study the functions of nucleic acids containing thio bases.

Nitrous acid has been widely used in biochemical studies of nucleic acids. It causes mutagenesis by bringing about deamination, or by producing deletions in DNA (1). It also causes covalent cross links between DNA strands or between protein and DNA (2, 3). This reagent has also provided a means to study the base sequences of DNA (4). Deamination with nitrous acid has been used in the modification of tRNA (5-9). We wish to report here a new reaction of nitrous acid the desulfurization of 4-thiouridine. This reaction occurs more rapidly, at least in the monomer level, than the known deamination of nucleosides.

When 4-thiouridine was treated with dilute nitrous acid at pH 4.0, a gradual change in its UV spectrum was observed with increasing time of reaction, and the spectrum finally became the same as that of uridine (Fig. 1). The spectral change suggested the presence of bis(4-thiouridine) disulfide in the intermediate stages. Paper chromatographic analysis of the reaction mixture gave two new spots that showed behavior identical to bis(4-thiouridine) disulfide and uridine, respectively. When bis(4-thiouridine) disulfide was treated with nitrous acid under similar conditions, a spectral change indicative of the formation of uridine was observed. Uridine derived both from 4-thiouridine and from the disulfide

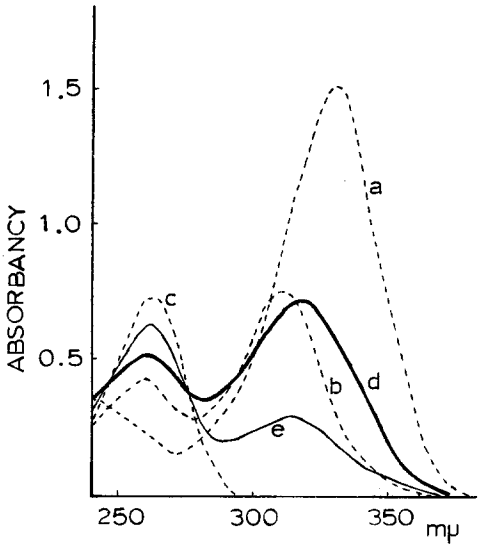



Fig. 1. A typical example of UV-spectral change of 4-thiouridine caused by nitrous acid. 3 mM 4-Thiouridine was treated with 18 mM sodium nitrite in 50 mM acetate buffer at pH 4.0 and 37°. At intervals, an aliquot (25 μl) was taken and added to 1 ml of 100 mM phosphate buffer, pH 7.2, and the spectrum of the resulting solution was determined; a, 4-thiouridine (0 time); b, bis(4-thiouridine) disulfide; c, uridine; d, aliquot at 4 hr; e, aliquot at 20 hr.

was identified by paper chromatography of the reaction mixture and by subsequent determination of the UV spectra (in acid and in alkali) of the spot having the same $R_F$ value as uridine. The possibility was excluded that this reaction might have been brought about by nitrate, which could have been present in the reaction mixture. Thus, 4-thiouridine was unaffected in 90 mM sodium nitrate at pH 4.0.

Rate of these reactions was compared with that of the deamination of guanosine, adenosine, and cytidine (Fig. 2). The rates were determined by a direct spectrophotometric method (10,11). Both at pH 4.0 and 4.9, the degradation of 4-thiouridine, as well as its disulfide, proceeded much more rapidly than the deamination of the nucleosides. At pH 4.9, the transformation of both 4-thiouridine and its disulfide was slower than at pH 4.0. It is to be noted that, at this pH, the accumulating amount of the disulfide derived from 4-thiouridine went up higher than that observed for pH 4.0-reaction. Little, if any, deamination was observed for the amino nucleosides at pH 4.9. The production of a small amount of 4-thiouridine from the disulfide appeared to be significant because in

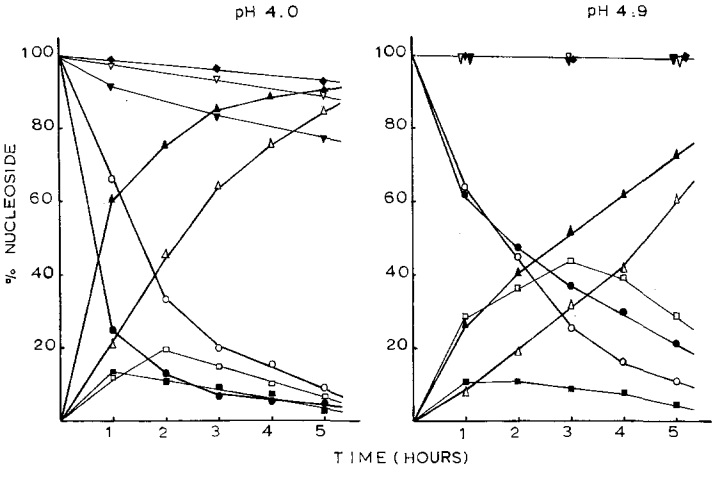


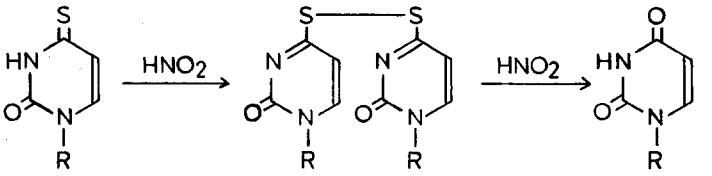

Fig. 2. Comparison of the rates of reaction between nucleosides and nitrous acid. 3 mM Nucleoside (1.5 mM for bis(4-thiouridine) disulfide) was treated with 90 mM nitrite in 200 mM acetate buffer at 37°. The amount of each component was determined by a direct spectrophotometric method (refs. 10 and 11). For 4-thiouridine and its disulfide, the absorbance was read at 260, 310, 320 and 335 mμ against an appropriate blank, containing buffer and nitrite. The molecular extinction coefficients of the thio compounds have been given previously (refs. 20 and 21). Products from 4-thiouridine are represented by, △ for uridine; □ for disulfide; and ○ for 4-thiouridine. Products from the disulfide are represented by, ▲ for uridine; ■ for 4-thiouridine; and ● for disulfide. Undeaminated nucleosides are represented by, ▼ for guanosine; ▽ for adenosine; and ◆ for cytidine.

the pH 4.0-reaction the $\lambda$ max of the disulfide at 310 mμ shifted to 318 mμ after 1 hr of the nitrous acid-treatment. At pH 7, little transformation of either 4-thiouridine or its disulfide by nitrite was observed. When 3 mM nucleoside was treated with 18 mM nitrite at pH 4.9 and 37° for 120 hrs, 4-thiouridine was quantitatively converted into uridine, while no deamination of guanosine was detected.

Similar results have been obtained by 1-methyl-4-thiouracil and its disulfide. In the reaction between 1-methyl-4-thiouracil and nitrite, bis(1-methyl-4-thiouracil) disulfide was isolated as a reaction product. Thus, 13 mM 1-methyl 4-thiouracil (106 mg) was treated with 65 mM sodium nitrite in 100 mM acetate buffer at pH 4.9 and 37° for 3 hrs, and the reaction mixture was cooled in an ice-bath for 1 hr. The resulting precipitate (21 mg) was collected and recrystallized from oxygen-free water; mp 230-231° (decomp.). The reported mp is 227°

(decomp.)(12). This compound was indistinguishable from the authentic bis(1-methyl-4-thiouracil) disulfide in terms of UV and IR spectra and paper chromatographic behavior. A preliminary experiment has shown that 2-thiouracil yields uracil on treatment with nitrous acid (13).

Nitrous acid thus brings about the reactions represented in Scheme 1.




Scheme 1

Of course, the intermediary formation of the disulfide may not be a prerequisite to the desulfurization of 4-thiouridine: There may exist a route by which 4-thiouridine is directly transformed into uridine. In this context, it should be noted that nitrous acid converts cystein into its disulfide through an intermediary formation of S-nitrosocystein (14). It is possible that such an unstable derivative is also participating in the reactions of nitrous acid with 4-thiouridine and with its disulfide. Both the formation of the dimer and the desulfurization may well proceed via such a common intermediate.

Previously known actions of nitrous acid on nucleosides are the deamination and the formation of nitro compounds (10, 15). E. coli tRNA, which contains thiouracil derivatives (16, 17) has often been treated with nitrous acid (6, 8), but the fate of the thio bases has not been reported. The present finding provides a note of caution for those using nitrous acid as a tool to study the functions of nucleic acids containing thio bases. It also adds a new way of desulfurization of 4-thiouridine to the list of many such methods previously reported (18-25).

Acknowledgement. Professor H. Uchida of University of Tokyo, Institute of

Medical Science, is gratefully thanked for his valuable discussions. His suggestions directed our attention to this project. The authors thank Mr. M. Inoue and Mr. M. Yano of our laboratory for their preparation of the substrates. Thanks are also due to Professor T. Ukita of our Faculty for his encouragement throughout this research.

References

(1) L. E. Orgel, Advances in Enzymol., 27, 289 (1965)
(2) E. P. Geiduschek, Proc. Natl. Acad. Sci., U. S., 47, 950 (1961)
(3) P. Dussault, J. M. Bourgault, and W. G. Verly, Biochim. Biophys. Acta, 213, 312 (1970).
(4) H. S. Shapiro, and E. Chargaff, Biochemistry, 5, 3012 (1966)
(5) W. Zillig, D. Schachtschabel, and W. Krone, Z. physiol. Chem., 318, 100 (1960)
(6) J. A. Carbon, Biochim. Biophys. Acta, 95, 550 (1965)
(7) J. A. Nelson, S. C. Ristow, and R. W. Holley, Biochim. Biophys. Acta, 149, 590 (1967)
(8) J. A. Carbon, and J. B. Curry, J. Mol. Biol., 38, 201 (1968)
(9) M. S. May, and R. W. Holley, J. Mol. Biol., 52, 19 (1970)
(10) R. Shapiro, and S. H. Pohl, Biochemistry, 7, 448 (1968)
(11) H. S. Loring in "The Nucleic Acids", Vol. I, E. Chargaff, and J. N. Davidson, Ed., New York, N. Y., Academic Press, 1955, p 191
(12) B. C. Pal, M. Uziel, D. G. Doherty, and W. E. Cohn, J. Am. Chem. Soc. 91, 3634 (1969)
(13) S. Iida, unpublished results
(14) U. Schulz, and D. R. McCalla, Canadian J. Chem., 47, 2021 (1969)
(15) H. Schuster, and G. Schramm, Z. Naturforsch., 13 B, 697 (1958)
(16) M. N. Lipsett, J. Biol. Chem., 240, 3975 (1965)
(17) J. A. Carbon, L. Hung, and D. S. Jones, Proc. Natl. Acad. Sci., U. S., 53, 979 (1965)
(18) K. Burton, Biochem. J., 104, 686 (1967)
(19) K. H. Sceit, Biochim. Biophys. Acta, 166, 285 (1968)
(20) E. B. Ziff, and J. R. Fresco, J. Am. Chem. Soc., 90, 7338 (1968)
(21) M. Yano, and H. Hayatsu, Biochim. Biophys. Acta, 199, 303 (1970)
(22) H. Hayatsu, J. Am. Chem. Soc., 91, 5693 (1969)
(23) H. Hayatsu, and M. Inoue, J. Am. Chem. Soc., in press
(24) R. T. Walker, and U. L. RajBhandary, Biochem. Biophys. Res. Commun., 38, 907 (1970)
(25) M. Saneyoshi, and S. Nishimura, Biochim. Biophys. Acta, 204, 389 (1970)