Heterocycles Related to Nucleotides. X. \(^1\) Reaction of Thiouridine with 2-Chloromercurio-4-nitrophenol

Eisuke SATO,*** Yuichi KANAOKA,** and Koichi KIMURA***

Faculty of Pharmaceutical Sciences, Hokkaido University,** Kita-12, Nishi-6, Kita-ku, Sapporo 060, Japan and Department of Applied Biological Sciences, Faculty of Science and Technology, Science University of Tokyo,*** Noda, Chiba 278, Japan

(Received February 7, 1990)

2-Chloromercurio-4-nitrophenol reacts reversibly with thiouridine, this reaction was applied to a chemical modification of E. coli transfer ribonucleic acid

**Keywords** — 4-thiouridine; 2-thiouridine; mercury dichloride; 2-chloromercurio-4-nitrophenol; chemical modification; transfer RNA

In the course of studies on chemical modification of thiouridine, we have reported the reaction of thiouridine with 2-hydroxy-5-nitrobenzyl bromide (Koshland reagent).\(^2\) In the reaction with Koshland reagent, thiouridine can not be regenerated. In many cases of chemical modification, reversible modification is desirable.

In this paper, we describe the reversible reaction of thiouridine with 2-chloromercurio-4-nitrophenol (2-CM-4-NP, 2b),\(^{3b-d}\) which was recently applied to a chemical modification or a chromophoric probe for SH enzyme.\(^{3b-d}\) Several examples have been reported on the reaction of thiopyrimidinone with mercurial reagents in the way of syntheses of thiopyrimidinone nucleoside as a protecting group of sulfur site.\(^4\)

Several examples of transfer ribonucleic acid (t-RNA) treated with mercury compound to titrate or modify 4-thiouridylic acid have presented,\(^5\) but there are no example of reversible reporter group of mercury compound. The reaction with mercury dichloride was examined as simplified model of reagent, prior to a reaction with 2-CM-4-NP (2b). Although dissociation of

---

\[ \text{S} \quad \text{NH} \quad + \quad \text{HgCl}_2 \quad \rightarrow \quad \text{S-HgX} \quad + \quad \text{HCl} \]

\[ \text{1} \quad \text{2} \quad \text{3} \quad \text{4} \quad \text{5} \]

\(a: X = \text{Cl}, \ R = \beta\text{-ribosyl} (3, \ 5: X = \text{thiouridine-S-yl})\)

\(b: X = 2\text{-}(4\text{-nitrophenol}), \ R = \beta\text{-ribosyl}\)

---

* To whom correspondence should be addressed.
TABLE I. pH or Concentration of Sodium Chloride or Ratio of Concentration at 50% Dissociation of Adducts

<table>
<thead>
<tr>
<th>Adduct</th>
<th>HCl</th>
<th>pH</th>
<th>NaOH</th>
<th>NaCl (m)</th>
<th>Ratio(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Thiouridine: HgCl(_2) (3a)</td>
<td>2.1</td>
<td>13.9</td>
<td>1.0</td>
<td>2 \times 10^4</td>
<td></td>
</tr>
<tr>
<td>2-Thiouridine: HgCl(_2) (5a)</td>
<td>2.9</td>
<td>12.2</td>
<td>0.05</td>
<td>1 \times 10^1</td>
<td></td>
</tr>
<tr>
<td>4-Thiouridine: 2-CM-4-NP (3b)</td>
<td>2.6</td>
<td>11.7</td>
<td>0.7</td>
<td>1 \times 10^4</td>
<td></td>
</tr>
<tr>
<td>2-Thiouridine: 2-CM-4-NP (5b)</td>
<td>3.7</td>
<td>10.2</td>
<td>0.02</td>
<td>4 \times 10^2</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Ratio of concentration of sodium chloride vs. concentration of thiouridine at 50% dissociation of adducts.

4-thiouridine mercury adduct by hydrogen chloride has reported by Ueda,\(^{46}\) further details was examined. Based on changes of ultraviolet spectrum pattern on hydrogen chloride concentration, 4-thiouridine mercury dichloride adduct (3a) was dissociated half at pH 2.1 to 4-thiouridine (1a) and mercury dichloride. The higher the concentration of hydrogen chloride, the more adduct is dissociated as shown in Fig. 1. It was also found that 4-thiouridine mercury dichloride adduct (3a) was dissociated not only with hydrogen chloride but also with sodium hydroxide or sodium chloride in high concentration. 2-Thiouridine mercury dichloride adduct (5a) can also be dissociated with hydrogen chloride, sodium hydroxide or sodium chloride. These results are summarized in Table I.

As shown in Table I, 2-thiouridine mercurials are dissociated more easily than 4-thiouridine mercurials with hydrogen chloride, sodium hydroxide or sodium chloride. Compared with mercury dichloride, 2-CM-4-NP tends to dissociate from 4- or 2-thiouridine with a lower concentration of hydrogen chloride, sodium hydroxide or sodium chloride. 4-Thiouridine 2-CM-4-NP adduct (3b) is stable in the range of pH 4—10, a region which is mostly used for biopolymer studies, so that this reagent 2-CM-4-NP might be promising as a reversible reporter group for polynucleotides containing 4-thiouridine. Table I (last column) shows the comparison of the bonding of mercury between thiouridine and chloride anion. For example, the bonding between 4-thiouridine and mercury is 2 \times 10^4 times stronger than that of chloride anion to mercury. Comparing the dissociation of thiouridine mercurial with hydrogen chloride and sodium chloride, indicates a marked tendency to dissociate more easily with hydrogen chloride. It can be suggested that the protonation on the N\(_3\)-position of thiopyrimidinone weakens the bond between sulfur and mercury permitting greater dissociation.

4-Thiouridylic acid is located in common position between dihydrouridine loop and 5'-terminal, so unfraccionated *Escherichia coli* t-RNA (Q-13 stump) was subjected to the reac-

Fig. 2. Dissociation of 3a vs. pH

![Graph showing dissociation of 3a vs. pH](image)

Fig. 3. Spectra on the Reaction of t-RNA (*E. coli.*) with Mercury Dichloride (2a) or 2-CM-4-NP (2b)

1: t-RNA (*E. coli.*) in Tris-HCl buffer. 2, 3, 4, 5, 6: t-RNA with 1, 2, 3, 4, 5 eq. of 2a, respectively. 7: t-RNA with 2b at pH 10 after dialysis.
tion with mercurials. As shown in Fig. 3, the spectral changes of t-RNA treated with mercury dichloride, indicates that four equivalents of mercury dichloride is need to change it fully. The t-RNA treated with four equivalents mercury dichloride, was further treated with mercaptoethanol or potassium cyanide. The initial spectrum was restored almost completely, and restored 14% with aq. sodium chloride (1 M). The solution of t-RNA with four equivalents of 2-CM-4-NP was dialyzed against Tris-HCl buffer. Four dialyses removed excess reagents up to 1.2 eq. Further dialysis excluded 2-CM-4-NP very slowly, and dialysis against water removed little reagent. 2-CM-4-NP can be removed very easily and the initial spectrum is restored by dialysis against aq. potassium cyanide (1 mM) or aq. mercaptoethanol (1 mM). These results are consistent with the reports of Pal50 or Jones.51

As a result, fundamental characteristics on 4- and 2-thiouridine mercurials were examined in detail, and the introduction of a reversible reporter group (2-CM-4-NP) to the 4-thiouridine moiety of t-RNA was elucidated. These results might be useful for study of nucleic acids containing 4-thiouridylic acids.

Experimental

**Instruments** — Melting points were determined on a Yamato MP-21 apparatus and are uncorrected. Ultraviolet and visible spectra were recorded on a Shimazu UV-200 spectrophotometer. pH was measured with a Hitachi-Horiba M-5 pH meter.

**4- and 2-Thiouridine (1a and 4a) —** Prepared according to Ueda’s procedure,69 1a: mp 134—135 °C (lit.,68 mp 135—138 °C), 4a: mp 213—214 °C (lit.,70 mp 205—207 °C).

**2-Chloromercuro-4-nitrophenol (2-CM-4-NP, 2a) —** Prepared with McMurray’s procedure,22 2a: mp 240—245 °C (lit.,22 mp 238 °C).

**Spectrometric Measurement for 3a, b and 5a, b** — 0.5 ml of 1a (1 mM, water) or 4a (1 mM, water), and 0.25 ml of 2a (1 mM, water) or 0.5 ml of 2b (1 mM, 0.005 N NaOH) were combined, then diluted to 10 ml (5 × 10⁻⁵ M) with HCl aq., NaOH aq., or H₂O with NaCl, and then ultraviolet and visible spectra were recorded. For example, spectral changes of adduct 3a vs. pH values (by using HCl aq.) were recorded (pH values were measured with a pH meter for each spectrum) as shown in Fig. 1. Based on the percent increase of optical density at 332 nm (percent dissociation of adduct) vs. pH in Fig. 2, pH of 50% dissociation of 3a to 1a and 2a was estimated at 2.1. Other cases of 3a with NaOH or NaCl, 3b, 5a and 5b were not shown, but other data in Table I were obtained by the same procedure with 3a and HCl. Differences of optical density were recorded at the following wave lengths (nm): 318 (NaOH) and 332 (NaCl) for 3a; 274 (HCl), 270 (NaOH) and 274 (NaCl) for 5a; 330 (HCl), 340 (NaOH) and 329 (NaCl) for 3b; 280 (HCl), 270 (NaOH) and 274 (NaCl) for 5b. Ratio in the last column of Table I were calculated by dividing the NaCl concentration at the 50% dissociation of adducts by the concentration of thiouridines (5 × 10⁻⁵ M).

**Reaction of t-RNA with Mercury Dichloride (2a) or 2-CM-4-NP (2b) —** To 4 ml solution (OD = 0.395 at 335 nm) of unfractionated transfer RNA (E. coli) in Tris-HCl buffer (10 mM, pH 7.6, MgCl₂ 0.1 mM), 5 μl each of 2a (0.08 μmol, 1 eq. assuming 2.0 × 10⁴ for molecular extinction coefficient of 4-thiouridylic acid residue) was added for ultraviolet spectrum measurement. As shown in Fig. 2, based on the decrease of optical density at 338 nm, addition of 1, 2, 3 and 4 equivalents of 2a modified 63, 82, 92 and 100%, respectively, of 4-thiouridylic acid residue of t-RNA. To the same solution (4 ml) of t-RNA, 4 equivalents of 2a was added and then 234 mg of NaCl (1M in 4 ml), 5 μl of 0.8 M KCN or 5 μl of 0.8 M mercaptoethanol was added, then ultraviolet spectra were measured. Although spectra were not shown, 14% of recovery with NaCl and almost complete recovery with KCN or mercaptoethanol were observed by the increase of optical density at 338 nm. To the same solution (4 ml) of t-RNA, 20 μl of 2b (4 eq.) were added, then dialyzed against 4 × 500 ml of Tris-HCl buffer (10 mM, pH 7.6, MgCl₂ 0.1 mM), and then ultraviolet and visible spectra were recorded at pH 10 (OD = 0.385 at 408 nm). As shown in Fig. 3, 1.2 equivalent of 2b versus 4-thiouridylic acid residue was introduced assum-
ing \(1.74 \times 10^4\) for molecular extinction coefficient \((e)\) of \(2b\) at 405 nm (pH 10) as judged by optical density at 405 nm.

Acknowledgement We are grateful to Professor Tohru Ueda (Faculty of Pharmaceutical Sciences, Hokkaido University) for his advice.

References


