

( $c=0.2$ , DMF),  $R_f^1$  0.88,  $R_f^2$  0.92, single ninhydrin-positive spot. *Anal.* Calcd for  $C_{162}H_{203}N_{21}O_{41}$ : C, 62.75; H, 6.60; N, 9.49. Found: C, 62.89; H, 6.69; N, 9.21.

**H-Lys-Asp-Leu-Lys-Glu-Lys-Lys-Glu-Val-Val-Glu-Glu-Ala-Glu-Asn-OH (VI)**—The protected pentadecapeptide V (50 mg) was treated with TFA (1 ml)-anisole (0.1 ml) at room temperature for 40 min, then dry ether was added. The resulting powder was washed with ether and dried over KOH pellets *in vacuo*. The de-Boc peptide ester was hydrogenated in 60% AcOH (15 ml) over 10% Pd-C for 20 hr. The catalyst was removed with the aid of cellite. The filtrate was evaporated to dryness and the residue was dried over KOH pellets *in vacuo*. The hydrogenated product was dissolved in a small amount of the upper phase of BuOH-AcOH-H<sub>2</sub>O (4:1:5). The solution was applied to a column of Sephadex G-25 (2.6 × 96 cm) previously equilibrated with lower phase of the above solvent system. The column was developed with the same upper phase. Individual fractions (5 ml each) were collected and the absorbancy at 230 nm was determined. The fractions corresponding to the main peak (tube No. 78–91) were combined. The solvent was removed by evaporation and the residue was lyophilized from H<sub>2</sub>O to give a fluffy white powder; yield 13 mg (59%), mp 248–255° (dec.),  $[\alpha]_D^{25}$  –42.1° ( $c=0.1$ , 10% AcOH),  $R_f^1$  0.06,  $R_f^2$  0.15, single ninhydrin-positive spot. Amino acid ratios in the acid hydrolysate: Glu 4.51, Asp 1.69, Val 2.02, Ala 1.03, Leu 0.94, Lys 3.54 (average recovery 84%). Amino acid ratios in the AP-M digest: Glu 4.58, Asp 0.93, Val 2.01, Ala 1.01, Leu 0.89, Asn 0.88, Lys 3.56 (average recovery 86%).

**Effect of Thymosin  $\alpha_1$  Fragments on the Inhibition of T-Lymphocyte Transformation by Uremic Serum**—Peripheral blood lymphocytes were isolated in a Hypaque-Ficoll gradient<sup>17)</sup> for T-cell transformation. The cells were cultured in 0.2 ml of RPMI-1640 containing 0.02 ml of uremic serum in microtiter plates (Falcon—3040) and 0.02 ml (final 1  $\mu$ g/ml) of PHA was added, with 0.02 ml (100–200  $\mu$ g/ml) of thymosin  $\alpha_1$  fragment. Triplicate cultures of each combination of  $5 \times 10^5$  cells per well were incubated at 37° in a humidified atmosphere of 5% CO<sub>2</sub> in air for three days. Twenty-four hr before harvest, 1  $\mu$ Ci/ml of <sup>3</sup>H-thymidine was added per culture. The amount of thymidine incorporated into DNA was measured in a scintillator.

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## Modification of Cytosine Moieties of Nucleic Acids with Hydrogen Sulfide (Nucleosides and Nucleotides. XXXIV)<sup>1)</sup>

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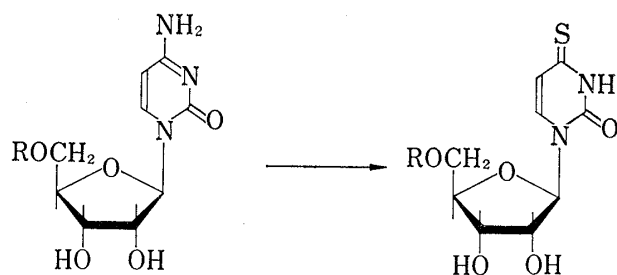
The chemical introduction of 4-thiouridine(S<sup>4</sup>U) residues into nucleic acids was carried out by the reaction of nucleic acids with liquid hydrogen sulfide in aqueous pyridine, which caused the conversion of cytosine moieties to 4-thiouracil moieties.

**Keywords**—chemical modification; sulfhydrolysis; hydrogen sulfide; 4-thiouridine; nucleic acids; yeast RNA; yeast tRNA; calf thymus DNA; ultraviolet absorption spectrum

It is important to investigate the chemical and physical properties of oligo- and polynucleotides containing 4-thiouridine (S<sup>4</sup>U) in order to obtain information on the function of the S<sup>4</sup>U residue in tRNA. Therefore, it seems desirable to be able to synthesize oligonucleotides containing S<sup>4</sup>U by a simple procedure.

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R=H or phosphate

Chart 1

We have reported an excellent method for the synthesis of S<sup>4</sup>U and its phosphate derivatives by means of an amino-thiol exchange reaction (sulfhydrolysis) of cytosine and its phosphate derivatives.<sup>3)</sup> This method has been successfully applied to prepare oligonucleotides containing S<sup>4</sup>U from oligonucleotides containing cytosine.<sup>4)</sup>

In this paper, the introduction of S<sup>4</sup>U residues into yeast RNA, torula yeast tRNA and calf thymus DNA by the application of sulfhydrolysis is described.

### Materials and Methods

Yeast RNA and calf thymus DNA were purchased from Sigma Chemical Co. and Miles Laboratories, Inc., respectively. Torula yeast tRNA was a gift from Jujo Pulp Co. Ltd., and had been prepared from *Torulopsis utilis* by the method reported by Miyazaki *et al.*<sup>5)</sup> The nucleotide composition of RNAs was determined by alkaline hydrolysis of RNA (20 mg) in 0.3 N KOH (2 ml) at 37° for 18 hr followed by column chromatographic separation of the products on Dowex 1 × 2 (Cl<sup>-</sup> form, 0.8 × 10 cm). Enzymic hydrolysis of tRNA and DNA and subsequent two-dimensional thin layer chromatography were carried out by the reported methods.<sup>6-8)</sup> Gel electrophoresis on 8% polyacrylamide gel slab was performed by the method of Ikemura *et al.*<sup>9)</sup>

**Sulfhydrolysis of Nucleic Acids**—A nucleic acid solution (50 mg of yeast RNA in 5 ml of H<sub>2</sub>O, 50 mg of tRNA in 25 ml of 0.15 M NaCl–0.015 M sodium citrate (pH 7.0), or 5 mg of heat-denatured calf thymus DNA in 5 ml of saline buffer) frozen at –70° in a stainless steel container was treated with liquid hydrogen sulfide solution (hydrogen sulfide gas was passed into 5 ml of pyridine at –70° until the volume reached 20 ml). The sealed container was heated at 40° for an appropriate time. After vaporization of most of the H<sub>2</sub>S the solution was concentrated *in vacuo* and water was added to the residue. The concentration procedure was repeated several times until pyridine was almost completely removed. The final turbid solution was applied to a column of Sephadex G-100 (1.6 × 24 cm) and elution was performed with 0.05 M KOAc (pH 6.0). RNA was recovered by combining the excluded fractions, followed by precipitation with ethanol overnight at –20°. The amount of RNA recovered was 40–42 mg. DNA was recovered by centrifugation of the turbid solution at 10000 rpm for 30 min followed by precipitation from the supernatant with ethanol. The yield was 3–4 mg.

### Results and Discussion

#### Sulfhydrolysis of Yeast RNA

Upon treatment of yeast RNA with liquid H<sub>2</sub>S in aqueous pyridine in a stainless steel tube at 40° for 60 hr, its UV spectrum showed an additional UV maximum at around 330 nm other than the maximum at 260 nm, with a ratio of  $A_{330\text{nm}}/A_{260\text{nm}}=0.22$ . This showed that the 4-thiouracil group had been introduced into the RNA. The presence of S<sup>4</sup>U in RNA treated with H<sub>2</sub>S was confirmed and the nucleotide composition was determined by alkaline

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hydrolysis of the RNA followed by chromatographic separation on a Dowex 1×2 column (Fig. 1). As is apparent in Fig. 1b, a new peak (V) having a UV absorption maximum at 330 nm appeared after the peaks corresponding to guanylates (IV). This material was identified as 2'(3')-4-thiouridylate on the basis of its UV spectrum and chromatographic behavior. The molar ratio of cytidylate (I) was decreased to 0.44 from 0.89 and the ratio of 4-thiouridylate formed was 0.36. The molar ratio of 4-thiouridylate was somewhat lower than was expected from the 50% decrease of cytidine residues. This may be due to a partial disulfide formation of 4-thiouridylate during the work-up procedure or the alkaline hydrolysis. The molar ratios of adenylate (II, 1.00), uridylate (III, 1.04) and guanylate (IV, 1.10) were unchanged after the reaction, which showed that the reaction occurred selectively at cytidine residues.

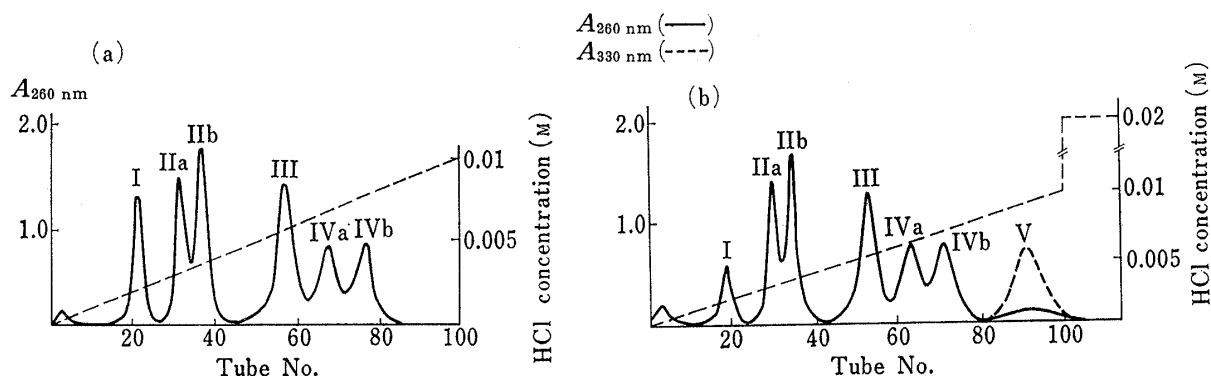


Fig. 1. Dowex 1×2 Column Chromatography of Hydrolysates of Untreated Yeast RNA(a) and  $\text{H}_2\text{S}$ -treated Yeast RNA (b)

**Sulphydrolysis of tRNA**

After treatment of yeast tRNA with  $\text{H}_2\text{S}$  at  $40^\circ$  for 12 hr, followed by digestion with ribonuclease M, 4-thiouridylate was detected by two-dimensional thin layer chromatography. The chromatographic patterns of the digests of untreated and  $\text{H}_2\text{S}$ -treated tRNA are illustrated in Fig. 2. A new spot corresponding to 4-thiouridylate ( $\text{S}^4\text{Up}$ , Fig. 2b) appeared after sulphydrolysis. As observed in the case of modified yeast RNA, the UV spectrum showed an absorption maximum at 330 nm with a ratio of  $A_{330\text{ nm}}/A_{260\text{ nm}}=0.07$ . The mean number of  $\text{S}^4\text{U}$  residues per molecule of tRNA modified with  $\text{H}_2\text{S}$  at  $40^\circ$  for 12 hr was calculated to be 2.7 based on the ratio of  $A_{330\text{ nm}}/A_{260\text{ nm}}$ , assuming the mean chain length of tRNA to be 80 and the number of cytidine residues per molecule to be 21. Polyacrylamide gel electrophoresis

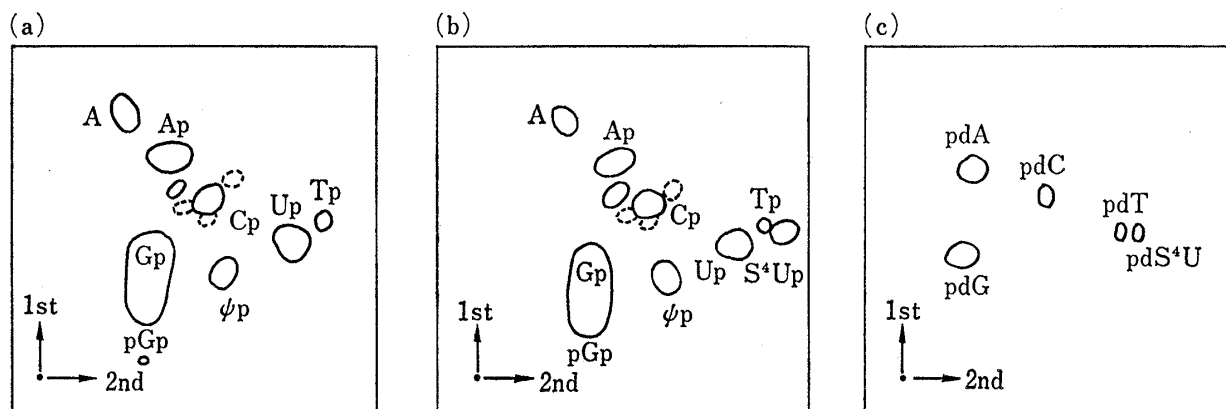
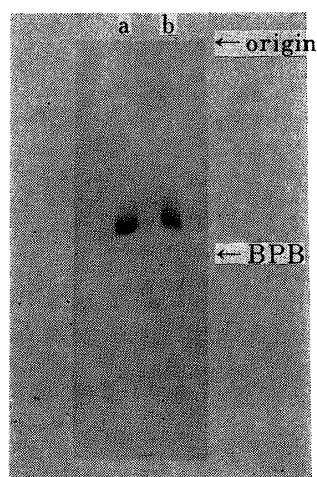


Fig. 2. Two-dimensional Thin Layer Chromatography of Ribonuclease M Digests of Yeast tRNA(a) and  $\text{H}_2\text{S}$ -treated tRNA(b) and of a Nuclease  $\text{P}_1$  Digest of  $\text{H}_2\text{S}$ -treated DNA(c)



a, untreated tRNA,  
b, H<sub>2</sub>S-treated tRNA  
BPB = Bromphenol blue

Fig. 3. Polyacrylamide Gel Electrophoresis of tRNAs

of the modified tRNA gave a single band, which showed that the sulfhydrolysis of tRNA proceeded without cleavage of the tRNA molecule (Fig. 3).

#### Sulfhydrolysis of DNA

When heat-denatured DNA was treated at 40° for 72 hr, the UV spectrum of the product showed the presence of 4-thiouracil groups ( $A_{330\text{nm}}/A_{260\text{nm}}=0.22$ ). The presence of 2'-deoxy-4-thiouridylate (pdS<sup>4</sup>U, Fig. 2c) was confirmed by nuclease P<sub>1</sub> digestion followed by two-dimensional thin layer chromatography. Thus, 4-thiouracil groups were also introduced into DNA by the sulfhydrolysis procedure.

Further studies on the sulfhydrolysis of tRNAs for specific amino acids and of certain 5S ribosomal RNAs are in progress in our laboratory.

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### Interactions of Sepharose-Bound Neurophysin I and II with Oxytocin and Vasopressin

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The interactions of Sepharose-bound bovine neurophysin I and II with oxytocin and arginine-vasopressin were studied.

The amounts of hormones bound to immobilized neurophysin II were found to depend strongly on temperature, being larger at 4° than at 36°.

It was found that oxytocin and vasopressin were eluted in different fractions from a column of Sepharose-neurophysin I by shallow pH gradients in either direction from pH 5.8, whereas the hormones emerged in the same fractions from a Sepharose-neurophysin II column.

**Keywords**—(bovine)neurophysin I; (bovine)neurophysin II; oxytocin; vasopressin; immobilized neurophysin; affinity chromatography

The bovine pituitary posterior lobe contains two main hormone-binding proteins, neurophysin I (NP-I) and neurophysin II (NP-II).<sup>2)</sup> Several studies have been carried out on the

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