5-(Carboxymethylaminomethyl)-2-thiouridine, a new modified nucleoside found at the first letter position of the anticodon

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ABSTRACT

The structure of a modified uridine derivative which was detected at the first letter position of the anticodon of *Bacillus subtilis* $tRNA_1^{Lys}$ was determined to be 5-(carboxymethylaminomethyl)-2-thiouridine. The determination was mainly based in its ultraviolet absorption spectra and mass spectrometric analysis of the trimethylsilyl derivative.

INTRODUCTION

We reported previously the nucleotide sequence of *Bacillus subtilis* $tRNA_1^{Lys}$ (1). It contained two unidentified modified nucleosides. One of them (K) located at the position following the 3'-end of the anticodon was recently identified as *N*-[(9-β-D-ribofuranosyl-2-methylthiopurin-6-yl)carbamoyl]threonine (2). Another one (U*) partly replaced the uridine residue located at the first letter position of the anticodon.

Here, we describe the identification of U* as 5-(carboxymethylaminomethyl)-2-thiouridine.

MATERIALS AND METHODS

Isolation of U nucleoside*

In the process of purification of ms^2t^6A from *B. subtilis* $tRNA_1^{Lys}$ previously described (2), the fragment containing U*, A-C-U-U(U*)-U-U-ms^2t^6A-A-Ψ-C-A-G- was isolated and the content of U* was 0.3 mole/mole of the fragment. By digestion of the fragment with RNase A and alkaline phosphatase, the U*-U fragment was separated by DE 23 (Whatman Inc.) column chromatography with a TEAB gradient. After U*-U was treated with excess amounts of RNase T_2 and alkaline phosphatase, the U* nucleoside (ca. 3 A_{260} units) was obtained by paper chromatography (Toyo filter paper 51 A, solvent system, isobutyric acid : 0.5 M NH_3, 5:3 by vol.).

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Mass spectrometry

The trimethylsilyl derivative of U* was prepared according to McCloskey (3). To 0.8 A_{260} unit of dried U* nucleoside, 4 µl of dry pyridine and 30 µl of *N-O*-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane were added. The mixture was heated for 1 hr at 100°C in a sealed glass tube. Low-resolution mass spectrum of trimethylsilylated U* was recorded using a JEOL JMS-01SG-2 instrument. The sample was introduced by direct inlet probe (ionizing energy 75 eV, probe templature 140-150°C).

RESULTS

UV-absorption spectra of U* (Fig. 1) showed remarkable resemblance to those of 5-(methylaminomethyl)-2-thiouridine (mnm^5s^2U) which was detected at the first letter position of the anticodon of *E. coli* $tRNA^{Lys}$ (5-7). At pH 12, the spectrum showed an absorption maximun at 245 nm with a shoulder peak at 272 nm. This is a typical feature for 2-thiouridine derivatives. On two-dimensional chromatograms, however, the positions of U*p and U* (Fig. 2) were different from those of not only mnm^5s^2Up and mnm^5s^2U, respectively, but also

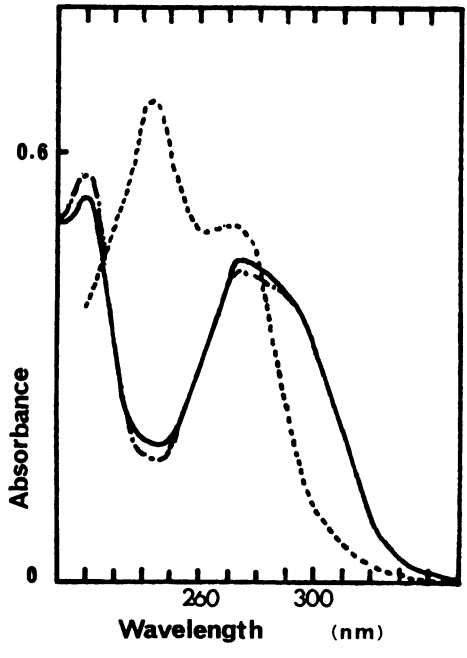


Fig. 1 Ultraviolet absorption spectra of U* nucleoside
(—·— pH 2, —— pH 6, ---- pH 12)

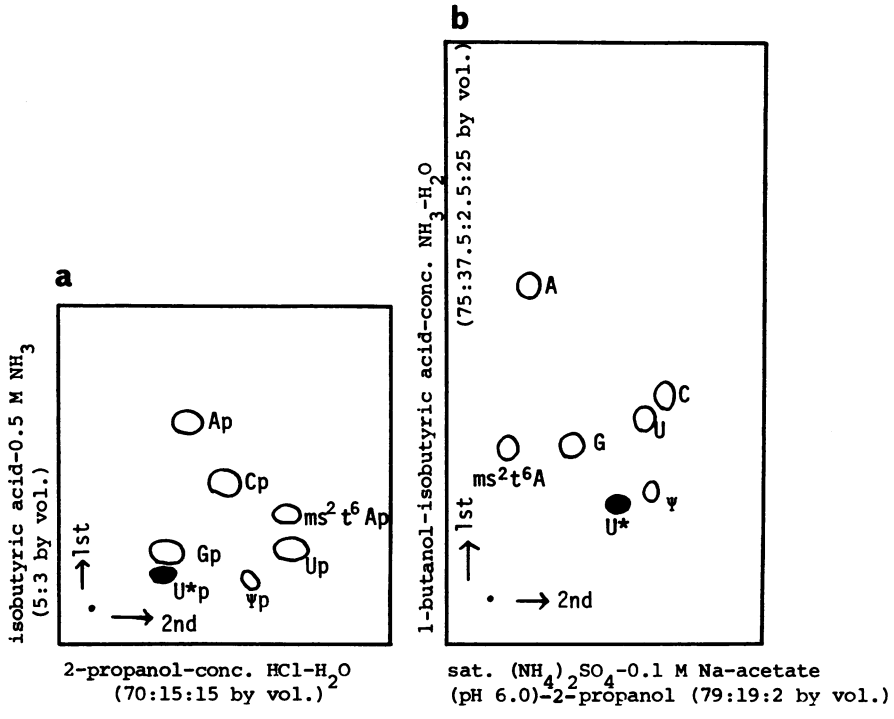


Fig. 2 Two dimensional thin layer chromatographies of U*p and U* nucleoside
a) Chromatogram of U*p on Avicel SF plate
b) Chromatogram of U* nucleoside on aluminum roll cellulose plate

any other known derivatives of 2-thiouridylate and 2-thiouridine, respectively (4,8). By DE 23 column chromatography with a TEAB gradient, the U* nucleoside was eluted more slowly than usual nucleosides. By electrophoresis on an Avisel SF plate in 0.05 M TEAB (pH 8.6), the U* migrated toward the anode (R_{Up}=0.45). These behaviors suggest that the U* has a negative charge. The ninhydrin test gave the U* a reddish-brown color with sensitivity comparable to sarcosine, an imino acid. These results indicated that the U* is a 5-substituted 2-thiouridine derivative and that the substituent at the 5-position contains an imino group as well as probably a carboxyl group.

Final identification of U* as 5-(carboxymethylaminomethyl)-2-thiouridine was given by mass spectroscopy. Fig. 3a shows the mass spectrum of trimethylsilylated U* (TMS-U*). The molecular ion peak of TMS-U* was detected at the position of mass number 635. When a comparison was made with mass spectra of TMS-U* and TMS-product of 5-(carboxymethylaminomethyl)uridine

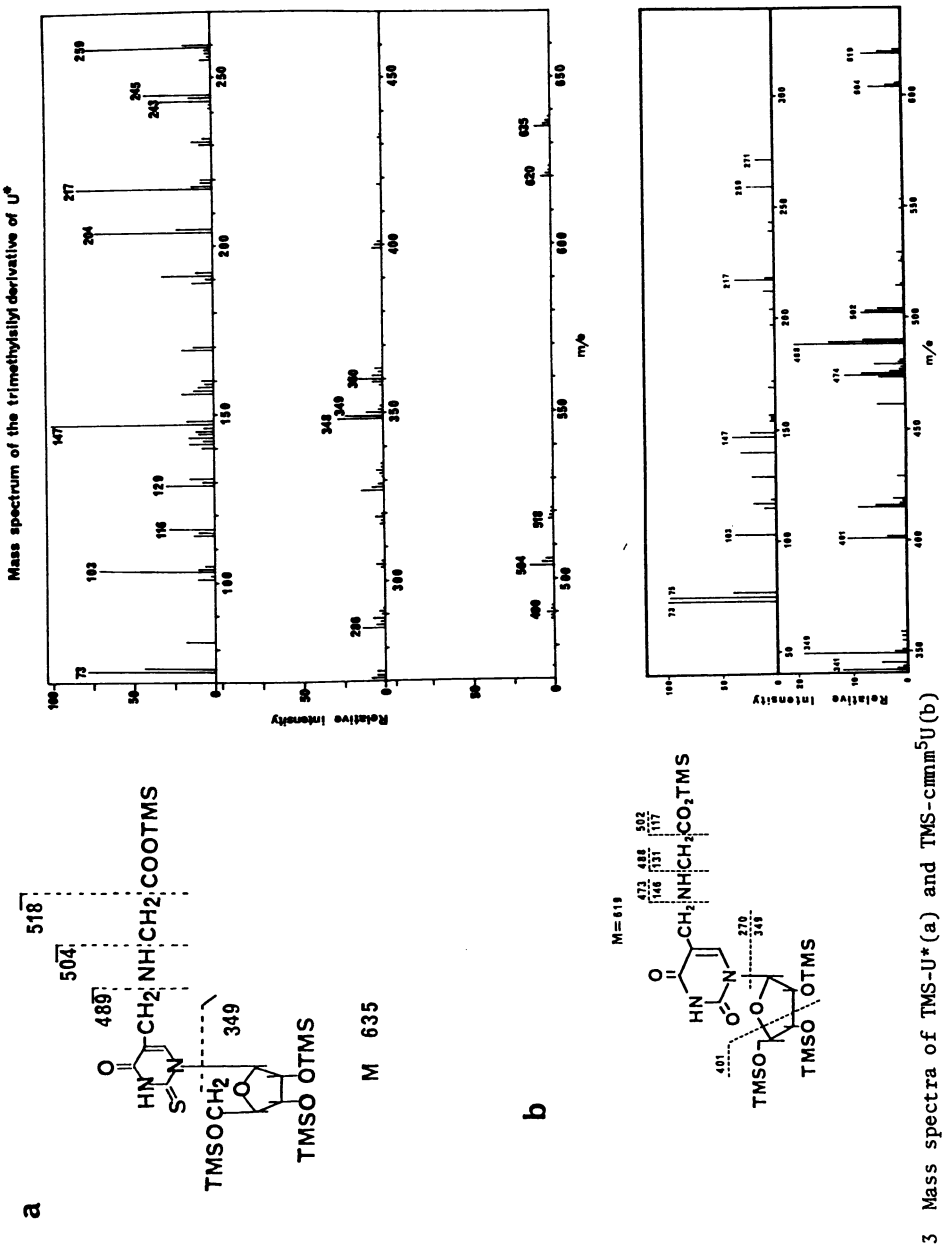


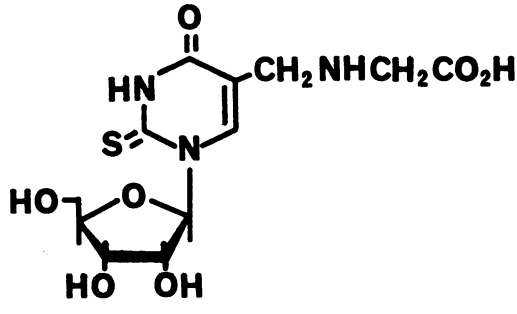
Fig. 3 Mass spectra of TMS-U*(a) and TMS-cmnm^{5}U(b)

(cmnm^{5}U) shown in Fig. 3b (9), this is 16 mass units more than that of the molecular ion peak of TMS-cmnm^{5}U. Mass numbers of all the main fragment ion peaks of TMS-U* were 16 mass units greater than those of the corresponding fragment ion peaks of TMS-cmnm^{5}U, i.e., 518, 504 and 490 are 16 more than 502, 488 and 474, respectively. The difference of 16 mass units can be explained by the replacement of an oxygen atom with a sulfur atom at the 2-position of uracil ring. This fragmentation pattern clearly demonstrates that the structure of U* is 5-(carboxymethylaminomethyl)-2-thiouridine as shown in Fig. 4.

By an attempt for desulfurization of the U* according to Saneyoshi and Nishimura (10), the U* gave three spots on a thin layer chromatogram developed with the same solvent systems as shown in Fig. 2a, although 2-thiocytidine was converted with a 100% yield to cytidine when treated under the same condition. One of the three spots, 20% of total products, was located at the same position as given by cmnm^{5}U (9). Its UV-absorption spectra quite resembled those of cmnm^{5}U at pH 2 and pH 12. We guess that 20% of cmnm^{5}s^{2}U was converted to cmnm^{5}U, while the rest were by-products.

DISCUSSION

The structure of a new compound detected in tRNA$_1^{Lys}$ from *B. subtilis* was determined to be 5-(carboxymethylaminomethyl)-2-thiouridine. Usually, 2-thiouridine derivatives located at the first letter of the anticodon show preferential affinity towards A of the third letter of the codons (11). It was reported that the removal of 2-thio group from mcm^{5}s^{2}U of yeast tRNA$_1^{Lys}$ re-



5-(Carboxymethylaminomethy)-2-thiouridine

Fig. 4 Chemical structure of U* nucleoside

duced the activity of protein synthesis (12). Unfortunately, the coding property of $cmnm^5s^2U$ was unable to be determined, because in our growing condition only a slight percentage of uridine located at the first letter of the anticodon was modified to $cmnm^5s^2U$. In fact, our $tRNA_1^{Lys}$ preparation showed an equal extent of stimulation in the binding to ribosome of lysyl-$tRNA_1^{Lys}$ by A-A-A and A-A-G (1).

When the 5-substituted uridines from *B. subtilis* are compared with those from *E. coli*, 5-methoxyuridine (mo^5U) is present in *B. subtilis* $tRNA^{Val}$, $tRNA^{Ala}$ and $tRNA^{Thr}$ (13), while uridin-5-oxyacetic acid (cmo^5U), a carboxylated form of mo^5U, is present in *E. coli* $tRNA^{Val}$, $tRNA^{Ala}$ and $tRNA_1^{Ser}$ (14) On the other hand, $cmnm^5s^2U$ detected in *B. subtilis* $tRNA_1^{Lys}$ is a carboxylated form of mnm^5s^2U present in *E. coli* $tRNA^{Lys}$, $tRNA^{Glu}$ and $tRNA^{Gln}$. Thus, in the two groups of codon-related uridine modification (mo^5U vs. cmo^5U : $cmnm^5s^2U$ vs. mnm^5s^2U), the carboxylation (or decarboxylation) patterns of the substituents at the 5-position of uracil ring are completely opposite between tRNAs from *B. subtilis* and *E. coli*. The meaning of this interesting finding must await further elucidation.

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Abbreviations: $cmnm^5s^2U$, 5-(carboxymethylaminomethyl)-2-thiouridine; $cmnm^5U$, 5-(carboxymethylaminomethyl)uridine; mo^5U, 5-methoxyuridine; cmo^5U, uridin-5-oxyacetic acid; mcm^5s^2U, 5-(methoxycarbonylmethyl)-2-thiouridine; mnm^5s^2U, 5-(methylaminomethyl)-2-thiouridine; TEAB, triethylammonium bicarbonate.

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