RESTRICTION OR AMPLIFICATION OF WOBBLE RECOGNITION

The structure of 2-thio-5-methylaminomethyluridine and the interaction of odd uridines with the anticodon loop backbone

W. HILLEN, E. EGERT, H. J. LINDNER and H. G. GASSEN
Institut fur Organische Chemie und Biochemie, Technische Hochschule Darmstadt, Petersenstr. 22, D-6100 Darmstadt, FRG

Received 17 July 1978
Revised version received 24 August 1978

1. Introduction

With few exceptions, nearly all tRNAs sequenced so far contain modified uridine derivatives instead of uridine in the first position of the anticodons [1]. Their occurrence is accompanied by a restriction or amplification of the wobble recognition [1,2]. One class of nucleosides consisting of V [3] and mo^5U [4] recognizes U in addition to A and G within a codon. The second class, namely s^2mcm^5U [5], s^2mnm^5U [6], and mcm^5U [7] forms base pairs with A and not with G. This restriction in base pairing was originally attributed to the presence of the 2-sulphur which was believed to prevent the formation of the G:s^2U base pair [8]. However, mcm^5U pairs exclusively with A [7] and from the crystal structures of ncm^5U [9] and s^2mcm^5U [10] it may be derived that the bulky substituent in the 5-position of the uracil moiety prevents the G:mcm^5U base pair formation via an interaction with the anticodon loop backbone.

Aside from s^2mnm^5U the crystal structures of all of these uridine derivatives have been described [9–12]. In this study we report, in brief, the crystal and molecular structure of $s^2mnm^5U \cdot 2\ H_2O$. Furthermore, we computed plots in which Gm (34) is replaced by the molecular structures of the different odd nucleosides taken from their crystal structures. The amplification or restriction of wobble recognition is discussed on the basis of possible interactions between the modified uridines and the anticodon loop backbone as derived from the crystal structure of yeast $tRNA^{Phe}$ [13].

Abbreviations: s^2mnm^5U, 2-thio-5-methylaminomethyluridine; s^2mcm^5U, 2-thio-5-methylcarboxymethyluridine; mo^5U, 5-methoxyuridine; V, 5-oxyaceticaciduridine; mcm^5U, 5-methylcarboxymethyluridine; ncm^5U, 5-carbamoylmethyluridine; Gm, 2′-*O*-Methylguanosine

2. Materials and methods

A sample of s^2mnm^5U was a kind gift of Dr H. Vorbrüggen, Berlin. Crystals were grown from oxygen-free water by slowly cooling from 70°C to ambient temperature. The crystal data are given in table 1. The structure was solved using direct methods. The final *R* factor was 0.063. Details of the X-ray analysis will be reported elsewhere.

3. Results and discussion

Figure 1 displays the molecular structure of $s^2mnm^5U \cdot 2\ H_2O$. The nucleoside is in the zwitterionic form deprotonated at N (3) and protonated at the amino nitrogen N (51). The ribose exhibits the 3′-endo conformation and is *gauche–gauche* at O (5′). The water in the crystal forms channels of ice-like structures along the *c*-axis with the nucleoside moieties connected around it by means of two hydrogen bonds each. The nucleoside is in the anti-conformation ($\chi_{CN} = 15°$) which is stabilized by the charac-

Table 1
Crystal data of $s^2mnm^5U \cdot 2\ H_2O$

Molecular formula: $C_{11}H_{21}N_3O_7S$

M = 339 g . mol^{-1}
Space group: $P2_12_12_1$
a = 13.695 (7) Å
b = 11.364 (6) Å
c = 9.748 (5) Å
V = 1517.1 Å^3
Z = 4
ρ_{obs} = 1.49 g . cm^{-3} (flotation)
ρ_{calc} = 1.484 g . cm^{-3}
1010 reflections measured
Structure determination by direct methods
Anisotropic refinement
Final R index: 0.063

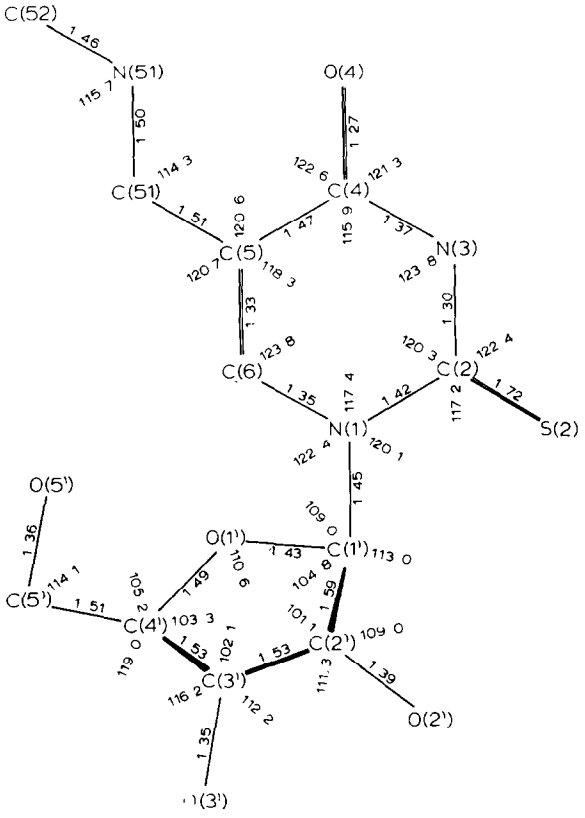

Fig.1. Molecular structure of $s^2mnm^5U \cdot 2\ H_2O$ showing (a) the conformation with the hydrogen bonds dashed and (b) the bond lengths and angles with standard deviations of 0.01 Å and 0.8°, respectively.

teristic intramolecular interaction O (5′) . . . H (C6). The 5-substituent forms an 8-membered ring including two hydrogen bonds to a water molecule: H (N51) . . . O (W) and H (OW) . . . O (4). This arrangement may reduce the ability of O (4) to form hydrogen bonds to other nucleosides.

If one evaluates the known molecular structures of wobble nucleosides, it is possible to consider the conformational reasons leading to the alterations in codon–anticodon recognition of the above-described uridine derivatives. Only the molecular structure of mo^5U reveals a possible explanation for the amplification of wobble recognition [12]. The elongated C (4)–O (4) and shortened N (3)–C (4) bond lengths are indicative of a shift of the keto-enol tautomerism of the 4-keto group. This result, however, has until now not been confirmed in molecular structures of other nucleosides. Therefore, we substituted Gm (34) with some wobble nucleosides by computer methods using the cartesian coordinates of the yeast $tRNA^{Phe}$ anticodon loop [13] in order to study their possible interaction with the anticodon loop backbone [9,10].

Figure 2 shows a stereo view of such a plot for s^2mnm^5U. The molecular structure as derived from the $s^2mnm^5U \cdot 2\ H_2O$ crystals is changed to a minor extent to obtain a nearly parallel stack of the anticodon bases (i.e., χ_{CN} is lowered to 0°C). A hydrogen bond from H (N51) – the amino nitrogen should be protonated in the tRNA molecule, too – to O (2′) of U (33) may hold the base moiety in this conformation. The O (2′)–H (N51) distance is ~2 Å and the angle O (2′) · · · H–N (51) is ~150°. If, however, the mnm-substituent is turned around C (5)–C(51) by 180°, a hydrogen bond between H (N51) and an oxygen from the phosphodiester moiety is possible and may result in almost the same fixed conformation of the wobble base. Similar results have been reported for ncm^5U [9] and s^2mcm^5U [10]. In the latter steric hindrance prevents the slight distortion necessary for the formation of the G:U pair. In the case of s^2mnm^5U this distortion is prevented by the hydrogen bond in each of the two possible conformations. It therefore appears that odd nucleosides showing a restriction of wobble recognition have bulky substituents in the 5-position which show a very strong interaction with the anticodon loop backbone.

Figure 3 shows a stereo view of mo^5U [12] plotted

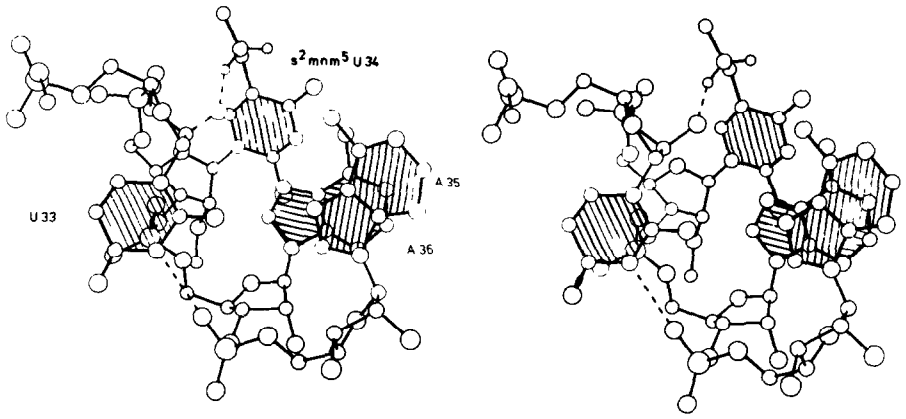

Fig.2. Stereo view of s^2mnm^5U plotted instead of Gm (34) in the anticodon loop structure of yeast $tRNA^{Phe}$. The possible hydrogen bond is indicated by a dashed line between H (N51) and O (2') of U (33).

in the wobble position of the $tRNA^{Phe}$ structure. Apparently, this nucleoside does not interact with the anticodon loop backbone. The methoxy group has been rotated around C (5)–O (5) by 180° to prevent steric hindrance of the methyl group with the phosphodiester between U (33) and the wobble nucleoside. This arrangement shows two characteristics:

(i) The wobble of this nucleoside, i.e., the conformational flexibility, is not hindered;

(ii) The methyl group is near O (4) and may protect it sterically which could result in a decreased specificity of hydrogen bonding.

The same reasoning may hold for the nucleoside V, the carboxylic group of which does not interact with the anticodon loop backbone. This group of nucleosides showing an amplification of the wobble recognition have the second atoms of the 5-substituent in the plane of the pyrimidine ring. This conformation which is favored by hyperconjugation, results in a more compact structure of these nucleosides showing no steric restriction of the wobble. Thus, the special

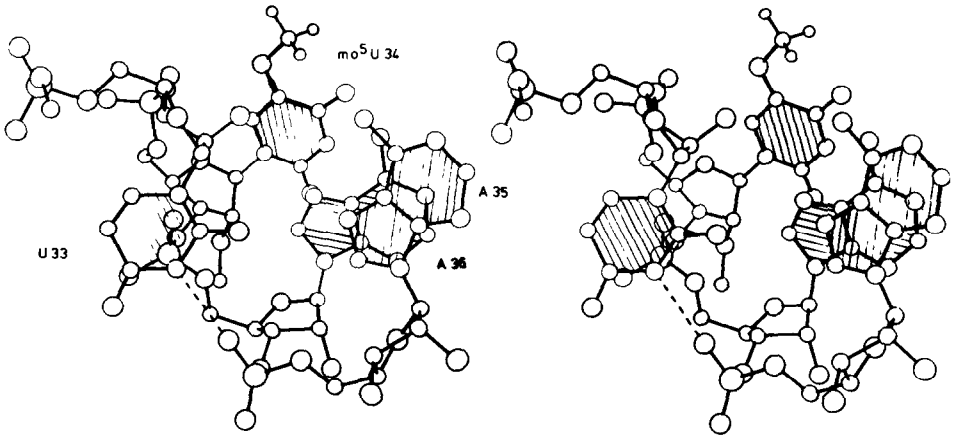

Fig.3. Stereo view of mo^5U plotted instead of Gm (34) in the anticodon loop structure of $tRNA^{Phe}$. The methyl group is turned by 180° around C (5)–O (5) which results in a conformation with the same stability and prevents steric hindrance with the anticodon loop backbone.

arrangement of modified uridines in the anticodon loop structure of tRNAs, assuming that the 'U-turn' is a general structural feature for all elongation-tRNAs, seems to explain their function in codon anticodon recognition on the basis of the originally postulated theory [2]. One group of substituents prevents wobbling in the first position of anticodons while the other allows the predicted conformational flexibility [2]. The substituents of the latter group may influence the keto-enol tautomerism of N (3)–C (4)–O (4) [12] which would reduce the specificity of base recognition as an intrinsic property of these nucleosides.

Acknowledgements

We are grateful to Mrs E. Rönnfeldt and Dr O. E. Beck for their help preparing the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft.

References

[1] McCloskey, J. A. and Nishimura, S. (1977) Acc. Chem. Res. 10, 403–410.
[2] Crick, F. H. C. (1966) J. Mol. Biol. 19, 548–555.
[3] Takemoto, T., Takeishi, K., Nishimura, S. and Ukita, T. (1973) Eur. J. Biochem. 38, 489–496.
[4] Murao, K., Hasegawa, T. and Ishikura, H. (1976) Nucl. Acids Res. 3, 2851–2860.
[5] Tumaitis, T. D. and Lane, B. G. (1970) Biochim. Biophys. Acta 224, 391–403.
[6] Chakraburtty, K., Steinschneider, A., Case, R. V. and Mehler, A. H. (1975) Nucl. Acids Res. 2, 2069–2075.
[7] Weissenbach, J. and Dirheimer, G. (1978) Biochim. Biophys. Acta 518, 530–534.
[8] Vormbrock, R., Morawietz, R. and Gassen, H. G. (1974) Biochim. Biophys. Acta 340, 348–358.
[9] Berman, H. M., Marcu, D., Narayanan, P., Fissekis, J. D. and Lipnick, R. L. (1978) Nucl. Acids Res. 5, 893–903.
[10] Hillen, W., Egert, E., Lindner, H. J. and Gassen, H. G. (1978) Biochemistry, in press.
[11] Morikawa, K., Torii, K., Iitaka, Y., Tsuboi, M. and Nishimura, S. (1974) FEBS Lett. 48, 279–282.
[12] Hillen, W., Egert, E., Lindner, H. J., Gassen, H. G. and Vorbrüggen, H. (1978) J. Carb. Nucleos. Nucleot. 5, 23–32.
[13] Quigley, C. G., Seeman, N. C., Wang, A. H., Suddath, F. L. and Rich, A. (1975) Nucl. Acids Res. 2, 2329–2341.