MD SIMULATION STUDIES TO INVESTIGATE ISO-ENERGETIC CONFORMATIONAL BEHAVIOUR OF MODIFIED NUCLEOSIDES M^2G AND M^2^G PRESENT IN tRNA

Rohit S Bavi a, Susmit B Sambhare a, Kailas D Sonawane a,b*

Abstract: Modified nucleic acid bases are most commonly found in tRNA. These may contain modifications from simple methylation to addition of bulky groups. Methylation of the four canonical nucleotide bases at a wide variety of positions is particularly prominent among the known modification. Methylation of N2 group of guanine is a relatively common modification in tRNA and tRNA. N2-methyladenosine (m2A) is the second most often encountered nucleoside in E. coli tRNAs. N2, N6-dimethylguanosine (m2G) is found in the majority of eukaryotic tRNAs and involved in forming base pair interactions with adjacent bases. Hence, in order to understand the structural significance of these methylated nucleic acid bases we have carried out molecular dynamics simulation to see the salvation effect. The results obtained shows iso-energetic conformational behaviors for m2G and m2^G. The simulation trajectory of m2G shows regular periodical fluctuations suggesting that m2G is equally stable as either s-cis or s-trans rotamers. The two rotamers of m2G may interact canonically or non-canonical with opposite base as s-trans m2G26:C/A/U44 and s-cis m2G26:A/U44. The free rotations around the C-N bond could be the possible reason for these isoenergetic conformations. Dimethylation of G has almost no influence on base pairing with either A or U. Thus, these results reveal that modified nucleosides m2G and m2^G may play an important role to prevent tRNA from adopting the unusual mitochondrial like conformation.

Research Article

Introduction

RNA molecules undergo extensive post-transcriptional modifications that are important for their biological activities. Post-transcriptional modifications have been known as a natural mechanism to provide structural stability across the wide range of temperature in archae as well as bacteria [1]. Transfer RNAs have the largest number and the greatest diversity of modifications; base or ribose methylation, base isomerization, base reduction, base thiolation and more complex hypermodifications [2, 3]. An important characteristic of tRNA is the presence of high content of modified nucleosides of which methylation represents the principle post-transcriptional modification during its maturation. In the maturation process of tRNA, transfer of methyl group occurs at polynucleotide level through an S-adenosyl-L-methionine donor, resulting in modification of heterocyclic base, the ribose moiety, or both [4]. The family of structurally related nucleosides m2G, m2A, m2Gm and m2^Gm, are from known archaeal tRNA sequences. These modified nucleosides are conserved at only two locations, position 10 first base in the proximal position of the dihydrouridine (DHU) arm and at position 26, junction between the D-stem and the anticodon stem, where they play crucial roles in the control and stabilization of the tertiary L fold structure of the tRNA [5, 6]. The m2G and m2^G modifications in tRNA are found not only at position 26 but also at positions 6, 7, 9, 10, 18 and 27 in various organisms [7].

Experimentally, it has been found that level of certain modified nucleosides in archael thermophiles play major stabilizing role beyond the effects of magnesium ion binding and G-C content of tRNA [8]. Earlier study involving three-dimensional models of yeast tRNAs derived from X-ray crystallographic data implies that m2G26 functions as a molecular hinge.

This hinge adjusts the angular position of the D-stem and the anticodon stem during protein synthesis, thus maintaining a certain rigidity/flexibility in this part of tRNA [9]. Nuclear magnetic resonance studies on the resonance of the methyl proton in yeast tRNAs also provide evidence to support the notation that m2G26 has a significant role in regulating the stacking and conformational dynamics of this region of tRNA molecule [10]. The yeast tRNA (m2G26) methyltransferase is dependent on the D-stem sequence and size of variable loop for the synthesis of N2,N2 dimethyl guanosine at 26th position [11]. Mutations were introduced in both the D-stem and the variable loop of tRNA105 to obtain dimethylation of the normally unmodified G26 by the yeast N2, N2-dimethyl G26-methyltransferase [12]. The presence of m2G26 in cytosolic tRNA may avert the molecule from adopting an unusual mitochondrial tRNA pattern folding and instead, allow it to fold into the canonical cloverleaf model. Through screening of the tRNA sequence and gene database it was revealed that some cytosolic tRNAs have the potential to fold into alternate structures. It was further noted that when a tRNA had the potential for this alternate folding, m2G was found at position 10 and 26 presumably to block the formation of this non-standard folding pattern [13]. The methylated guanosine from 26position of tRNA may have role in regulating the stacking interactions and the conformational dynamics [14].

N2-methylguanosine is found in both helical and loop regions of RNA secondary structure [15, 16] and it can exist in either s-cis or s-trans rotamers [18]. Incorporation of m2G was found to be isoenergetic with G in the duplex context as well as in GNRA (N = any

*Corresponding author. Tel.: +91 9881320719; Fax: +91 2312692333
E-mail address: kds_biochem@unishivaji.ac.in (Kailas D. Sonawane)

aStructural Bioinformatics Unit, Department of Biochemistry, Shivaji University, Kolhapur 416 004, Maharashtra, India
bDepartment of Microbiology, Shivaji University, Kolhapur 416 004, Maharashtra, India
The iso-energetic conformations of \( m^2G \) and \( m^2\text{G} \)

Results and Discussion

**Dynamic behavior of \( N^2\)-methylguanosine (\( m^2G \))**

The preferred conformation of \( N^2\)-methylguanosine \[18\] (Fig. 2A) has been used as a starting geometry for 20 ns molecular dynamics simulation study. In order to confirm the iso-energetic conformational behavior of \( m^2G \) we have analyzed four different average structures particularly at 0 to 1 ns (Fig. 2B), 3 to 4.5 ns (Fig. 2C), 5 to 11 ns (Fig. 3B) and 13 to 19 ns (Fig. 3C) and three snapshot structures particularly at 2 ns (Fig. 3A), 12 ns (Fig. 2D) and 20 ns (Fig. 2E) of 20 ns total simulation period. The geometrical parameters are mentioned in table 1. The selection of average and snapshot structures have been made based on the conformational flexibility observed during the MD simulation trajectory (Fig. 4, 6) similarly as per our earlier conformational studies of yW \[20\], OHyW \[21\] and ac\( ^\text{c} \)C \[32\].

**Stabilization of s-trans \( m^2G26 \) conformation**

The MD simulation average structures for \( m^2G \) taken at 0 to 1 ns (Fig. 2B), 3 to 4.5 ns (Fig. 2C), and snapshot structures at 12 ns (Fig. 2D) and 20 ns (Fig. 2E) shows the “proximal” or s-trans orientation with imidazole ring of guanosine.

The Methyl group of \( m^2G \) point towards the N(3) atom of guanosine as observed in earlier study \[18\]. This s-trans or proximal orientation would allow Watson–Crick base pair of \( m^2G26 \) with C44 and non Watson–Crick base pair with A/U44 at the hinge region of tRNA. Similar kind of s-trans orientation for \( m^2G \) has been observed in our earlier conformational study \[18\] along with crystal conformer of \( m^2\text{G10} \[33\], where it forms Watson–Crick base pairing with C25.

The average structure obtained at 0 to 1 ns (Fig. 2B) maintains the initial geometry (Fig. 2A) \[18\] by preserving s-trans or “proximal” conformation for \( N^2\)-methyl substituent of guanosine \( (26^a) \), which is stabilized by hydrogen bonding interaction between N(3)...H\( ^\text{C}(10) \) (Fig. 2B and Table 1). This average structure (Fig. 2B Table 1) shows deviations for torsion angle \( \beta \) by 87° and \( \chi \) by 155° whereas \( \alpha \) retains its initial geometry \[18\] as found in crystal conformer 1EHZ.pdb \[33\] and 6TNA.pdb \[9\]. A large deviation around the torsion angle \( \beta \) is due to rotations around C-N bond.
Next average structure taken at 3 to 4.5 ns (Fig. 2C) is also stabilized by hydrogen bonding between N(3)...HC(10) along with this, interaction between N(3)...HC2′ (Fig. 2C and Table 1) provides an additional structural stability to this average structure (Fig. 2C), as observed in our earlier conformational study of m^2G [18]. The torsion angle β shows large deviation (120°) whereas α maintains starting value as compared with initial structure of m^2G [18].

Snapshot structure selected at 12 ns (Fig. 2D) prefers s-trans or “proximal” conformation for m^2G and stabilized by N(3)...HC(10), N(3)...HC2′ and N(3)...HO2′ interactions (Fig. 2D and Table 1) similar to earlier results of m^2G [18]. This snapshot structure shows similar conformation for torsion angle α while torsion angles β and χ deviates to large extent from initial structure (Fig.2A) as observed in crystal structure 1OB5.pdb [34].

Second snapshot structure (Fig. 2E and Table 1) selected at final trajectory (20 ns) of simulation study also preserves s-trans conformation for m^2G and stabilized by intramolecular interactions between N(3)...HC(10), N(3)...HC2′ and O5′...HC(8) similar to the starting geometry (Fig. 2A) and PCILO preferred conformation of m^2G obtained without glycosyl torsion angle rotation (χ=16) [18]. Hence, this s-trans conformation of m^2G would form canonical Watson-Crick base pairing interaction with C44 and non-canonical Watson-Crick base pairing with A/U44 in order to provide structural stability to the tRNA molecule during protein biosynthesis process similarly as observed in earlier conformational and sequence analysis studies [18].

**Stabilization of s-cis m^2G26 conformation**

The MD simulation snapshot structure selected at 2 ns (Fig. 3A), and average structures at 5 to 11 ns (Fig. 3B) and 13 to 19 ns (Fig. 3C) shows s-cis orientation for methyl substituent of m^2G which point towards the N(1) atom of guanosine.

This orientation of N^2-methylguanosine allows non Watson-Crick base pairing with adenosine (A44) and uracil (U44) instead of usual Watson-Crick base pairing with cytosine (C44) at the hinge region of tRNA. This s-cis conformational behavior of m^2G was also noticed in our earlier study [18] and in rRNA crystal structure when m^2G is present at 10th position [34]. The usual Watson-Crick base pairing between m^2G10:C25 is not feasible when m^2G prefers s-cis orientation as observed in crystal conformer (PDB ID: 1OB5) [34] instead it would form other non Watson-Crick base pairing interactions with A44 and U44 at the hinge region of rRNA.

The geometrical parameters for torsion angles and hydrogen bonding interactions analyzed from average and snapshot structures are given in table 1. The snapshot structure for m^2G (Fig. 3A) taken at 2 ns prefers s-cis conformation due to change in α torsion angle which deviates from 180° to 29°, while other torsion angles β and χ diverges to great extent from initial structure and are in close agreement with crystal conformer 1OB5.pdb [34]. This structure is stabilized by the hydrogen bond between N(3)...HC2′ (Table 1) as found in earlier conformational study of m^2G [18]. Average structure (Fig. 3B) chosen for the period 5 to 11 ns when α torsion angle flipped by 179° as compared to preferred structure of m^2G (Fig. 2A).
Table 1. Geometrical parameters for torsion angles and hydrogen bonding interactions for average and snapshot structures after MD simulation.

<table>
<thead>
<tr>
<th>Modified nucleoside</th>
<th>Average structure at time (ns)</th>
<th>Torsion angle (degree)</th>
<th>Atoms involved (Atom 1 - Atom 2 - Atom 3)</th>
<th>Distance atom pair Atom1 - Atom 2 - Atom 3 (Å)</th>
<th>Angle Atom 1 - Atom 2 - Atom 3 (degree)</th>
<th>Figure Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>mG</td>
<td>PCIL0 most stable structure [18]</td>
<td>α=180°, β=60°, χ=286°</td>
<td>N(3)...H-C2' N(3)...H-C3'</td>
<td>1.992</td>
<td>117.02</td>
<td>2A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0-1</td>
<td>N(3)...H-C10</td>
<td>2.776</td>
<td>97.41</td>
<td>2B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>N(3)...H-C2'</td>
<td>2.503</td>
<td>125.19</td>
<td>3A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3-4.5</td>
<td>N(3)...H-C2'</td>
<td>2.898</td>
<td>103.42</td>
<td>2C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>05-11</td>
<td>N(3)...H-C1'</td>
<td>2.705</td>
<td>106.51</td>
<td>3B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>N(3)...H-O2'</td>
<td>2.946</td>
<td>158.15</td>
<td>2D</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13-19</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>N(3)...H-C10</td>
<td>2.838</td>
<td>93.97</td>
<td>2E</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>m2G</td>
<td>PCIL0 most stable structure [18]</td>
<td>α=0°, β=60°, γ=60°, χ=286°</td>
<td>N(3)...H-C2'</td>
<td>1.992</td>
<td>117.02</td>
<td>5A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-3</td>
<td>N(3)...H-C3'</td>
<td>2.269</td>
<td>113.78</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>19-20</td>
<td>O5’...H-C(8)</td>
<td>1.587</td>
<td>106.52</td>
<td>5C</td>
</tr>
</tbody>
</table>

The obtained average structure maintains distal conformation for methyl substituent of guanosine and gets stabilized by N(3)...HC1' interaction which was not observed in s-trans conformer of mG. Last average structure (Fig. 3C) was taken within the range of 13 to 19 ns, showing s-cis conformation of m2G. The s-cis conformation is obtained due to change in α torsion angle from 180° to 355°. This average structure (Fig. 3C) shows deviations for torsion angle β by 116° and χ by 73°. Obtained average structure (Fig. 3C) shows similar values for torsion angle α and χ as compared with crystal structure 1OB5.pdb [34]. A large deviation around the torsion angle β is due to fluctuations from s-trans to s-cis conformation by rotating C-N bond of methyl group.

Fluctuations in torsion angles of N2-methylguanosine (m2G) during MD simulation

Analyses were also made for torsion angles and hydrogen bonding interactions of mG during 20 ns simulation period (Fig. 4). The torsion angle α fluctuates periodically between s-trans (±180°) and s-cis (0°) rotamers of mG during total simulation period (Fig. 4A and Table 1). For simulation time 0 to 1 ns, 2.8 to 4.7 ns, 11.5 to 12 ns and 20 ns (Fig. 4A) torsion angle α prefers s-trans orientation which is supported by weak interaction between N(3)...HC2' (Fig. 4D) and N(3)...HC10 (Table 1). Orientation of α torsion angle favors the usual Watson-Crick base pairing of mG26 with C44 and unusual non-Watson-Crick base pairing with A/U44. Whereas, during simulation period 1 to 2.8 ns, 4.8 to 11.2 ns and 12 to 19.8 ns methyl substituent of guanosine prefers s-cis orientation which is stabilized by N(3)...HC2' and N(3)...HC1' hydrogen bonding interactions.

The s-cis conformation of N2-methyl substituent has also been observed in crystal structure when mG present at 10th position in tRNA[α] [34]. This orientation allows the non Watson-Crick base pairing between s-cis mG with A/U44. Similar results were found in earlier conformational energy calculations performed over mG [18].

Torsion angle β (Fig. 4B) maintains starting geometry ±180° [18] with small fluctuations at ±60° as found in crystal structure 6TNA.pdb [9], 1EHZ.pdb [33] and 1OB5.pdb [34]. The glycosyl torsion angle (χ) (Fig. 4C) fluctuates at ±30°, ±120°, ±180° and favors the respective anti (1.8 to 9.2 ns, 11.2 to 18.9 ns and 19.2 to 20 ns) and syn (0 to 1.7 ns, 9.3 to 10.2, 10.8 to 11 ns and 19 ns) conformation for mG. The mG is preferably stable at both syn and anti conformation, which allows usual (Watson-Crick) as well as unusual (non Watson-Crick) base pairing with C/A/U44. The syn conformation of s-trans and s-cis mG is supported by O5’...HC(8) hydrogen bonding interaction whereas in anti conformation of s-trans and s-cis mG, it is held by N(3)...HC2' and N(3)...HC(10) (Table 1) during MD simulation. Hydrogen bonding between O5’...HC(8) is varied in accordance with the fluctuations found in glycosyl torsion angle (χ) of mG26 during simulation period.
Molecular dynamics (MD) simulation study of \( N^2 - N^2 \) dimethyl guanosine (\( m^2G \))

In order to see solvation effect on \( N^2 - N^2 \) dimethylguanosine explicit molecular dynamics simulation study of 20 ns has been performed over the PCILO preferred conformation (Fig. 5A) [18]. To confirm the conformational behavior of \( m^2G \) we have analyzed two different average structures taken at 2-3 ns (Fig. 5B) and last 1000 ps from 19-20 ns (Fig. 5C), their geometrical parameters are listed in table 1.

The average structure obtained from 2 to 3 ns (Fig. 5B) prefers distal conformation for \( m^2G \) and prevent Watson-Crick base pairing with C, instead it would prefer non-canonical Watson-Crick interactions to pair with A/U44. Compared with crystal conformer (1EHZ.pdb) [33], average structure retains quite similar torsion angle values for \( \alpha, \beta \) and \( \chi \). The average structure selected at last 1000 ps (19 to 20 ns) does not show much difference as compared with earlier average structure. The only difference between these two average structures is variation around \( \alpha \) torsion angle which is positioned to 99° (Fig. 5C) from its preferred value (Fig. 5A). Due to this small change in conformational property of \( m^2G \), dimethylation of guanosine has almost no influence on pairing with either A or U, because the \( N^2 \) position of guanosine has no impact on these base pairing interactions. The \( m^2G \)-A pair can be formed with little hindrance, because, even though the methyl groups of \( m^2G \) are in the plane of the base, as in case of yeast tRNA\(^{\text{th}}\), they take part in a propeller-type arrangement with the base. The \( m^2G \)-U pair would not be affected by any conformational arrangement of the methyl groups similarly as discussed in [18].
The $\alpha$ torsion angle fluctuates periodically in between $\pm 180^\circ$ or $\pm 60^\circ$ (Fig. 6A and Table 1) over the 20 ns molecular dynamics simulation period suggesting free rotation around C-N bond.

Torsion angles $\beta$ (Fig. 6B) and $\gamma$ (Fig. 6C) retain preferred values $\pm 180^\circ$, with small fluctuations at $\pm 60^\circ$ throughout the simulation period. Glycosyl torsion angle ($\chi$) adopts anti conformation during the simulation study. Such type of anti conformation for N$^2$N$^2$-dimethylguanosine was confirmed through crystal structure (1EHZ.pdb, IEVV.pdb, 1OB5.pdb 6TNA.pdb).

**Conclusion**

The regular periodical fluctuations around the bond C(2)-N(2) of m$^2$G was observed throughout the 20 ns molecular dynamics simulation, which confirms the existence of iso-energetic s-cis or s-trans rotamers of m$^2$G. These iso-energetic rotamers interconvert.
easily during the simulation period. These results are in favor with preferred and alternative conformations of m^2G obtained by our earlier conformational energy calculations [18] as well as crystal structure (1EHZ.pdb [33] and 1OB5.pdb [34]). The periodical fluctuations of s-trans to s-cis and vice versa could be possible due to free rotations around the C-N bond of methyl group. According to tRNA sequence analysis [18] and this MD simulation results we would like to say that m^2G26 can form three different canonical as well as non-canonical Watson-Crick base pairing interactions with other bases. Such base pairing may be summarized as i) an usual Watson-Crick base pairing of m^2G26-C44 where the methyl substituent must be in s-trans orientation, ii) non-Watson-Crick base pairing between m^2G26-A where the methyl substituent is likely to be s-cis orientation, and iii) non-Watson-Crick m^2G26-U base pairing where the methyl group can adopt one of them, i.e. s-cis or s-trans conformation. These results reveal that m^2G is equally stable as either the s-cis or s-trans rotamers and the rotational preference of methyl group may be specific to the sequence context reliant upon which face of the base contributes in hydrogen bonding. Thus, MD simulation results confirm that the N^2-methyl group of m^2G20 may prefer energetically two stable rotamers, i.e., s-trans m^2G26:C/A/U44 and s-cis m^2G26:A/U44 as found in earlier results [18].

Similarly, the presence of two methyl groups unlike in case of single methyl in m^2G virtually eliminates the possibility of pairing with C and, indeed, m^22G26 pairs exclusively with A or U at position 44 and is flanked by C27G43 on one side and the m^2G10-C25-G45 triple on the other [35]. Hence, these results suggest that the modified nucleosides m^2G26 and m^22G26 play an important role in tRNA folding and may prevent tRNA from adopting the unusual mitochondrial like conformation.

Acknowledgements

Authors are gratefully acknowledged to University Grants Commission, New Delhi for financial support.

Citation

Bavi RS, Sambhare SB, Sonawane KD (2013) MD simulation studies to investigate iso-energetic conformational behaviour of modified nucleosides m^2G and m^22G present in tRNA. Computational and Structural Biotechnology Journal. 5 (6): e201302015. doi:http://dx.doi.org/10.5936/csbj.201302015

References