Mg²⁺ binding and archaeosine modification stabilize the G15–C48 Levitt base pair in tRNAs

ROMINA OLIVA,¹ ANNA TRAMONTANO,^{2,3} and LUIGI CAVALLO⁴

¹Dipartimento di Scienze Applicate, Università di Napoli “Parthenope,” I-80133 Naples, Italy

²Dipartimento di Scienze Biochimiche “A. Rossi Fanelli,” Università di Roma “La Sapienza,” I-00185 Rome, Italy

³Istituto Pasteur Fondazione Cenci Bolognietti, Università di Roma “La Sapienza,” I-00185 Rome, Italy

⁴Dipartimento di Chimica, Università di Salerno, I-84084 Fisciano (SA), Italy

ABSTRACT

The G15–C48 Levitt base pair, located at a crucial position in the core of canonical tRNAs, assumes a reverse Watson–Crick (RWC) geometry. By means of bioinformatics analysis and quantum mechanics calculations we show here that such a geometry is moderately more stable than an alternative bifurcated *trans* geometry, involving the guanine Watson–Crick face and the cytosine keto group, which we have also found in known RNA structures. However we also demonstrate that the RWC geometry can take advantage of additional stabilizing effects such as metal binding or post-transcriptional chemical modification. One of the few strong metal binding sites characterized for cytosolic tRNAs is localized on G15, and a domain-specific complex modification known as archaeosine is widespread at position 15 in archaeal tRNAs. We have found that both the bound Mg²⁺ ion and the archaeosine modification induce an analogous electron density redistribution, which results in an effective stabilization of the RWC geometry. Metal binding and chemical modification thus play an interchangeable role in stabilizing the G15–C48 correct geometry. Interestingly, these different but convergent strategies are selectively adopted in the different life domains.

Keywords: tRNA; tertiary interactions; metal binding; post-transcriptional modification; archaeosine

INTRODUCTION

Canonical tRNAs are characterized by an L-shaped conformation, made of two helical domains connected by nine tertiary base-pairing interactions (Fig. 1A). The 15–48 base pair, also known as the Levitt pair, is one of the crucial tertiary interactions in the core of cytosolic tRNAs. Located at the elbow of the L-shaped structure, it brings together the D and V arms, joining the two helical domains in tRNA molecules of both class I and II. The occurrence of a tertiary interaction between nucleotides 15 and 48 was already predicted in 1969 (Levitt 1969). Half a dozen years later the first structures for yeast tRNA^{Phe} revealed that the D and V loops run parallel in this part of the molecule (Kim et al. 1974; Robertus et al. 1974; Hingerty et al. 1978; Rich and Kim 1978; Sussman et al. 1978). Consequently, nucleotides 15 and 48 do not form a canonical Watson–Crick pair, but

assume a *trans* arrangement, known as reverse Watson–Crick (RWC) geometry (Burkard et al. 1999). In canonical tRNAs the 15–48 base pair is stacked between the 8–14–21 triplet and nucleotide 59 (Fig. 1A).

An interaction of the G–C type is found in 77% of canonical tRNAs at positions 15–48 (Sprinzl et al. 1998; Sprinzl and Vassilenko 2005), including the well-characterized yeast tRNA^{Phe}. The remaining nucleotide combinations are A–U (17%) and, more rarely, A–C and G–G, with the latter being a well-characterized identity element for *Escherichia coli* tRNA^{Cys} (Hauenstein et al. 2004). For yeast tRNA^{Phe} the G15–C48 base pair has been demonstrated to be required for a proper folding interaction to occur between the T and D domains (Nobles et al. 2002). Mutation of G15 and/or C48 nucleotides also severely affects the aminoacylation process of *E. coli* tRNA^{Pro} (Liu et al. 1995).

However, it is unclear whether the RWC geometry is stable for the G–C pair. Experimental studies on parallel strand DNA (psDNA) duplexes, characterized by RWC base pairs (Pattabiraman 1986; Mohammadi et al. 1998), have shown that, whereas the all A–T psDNA duplexes are quite stable, the introduction of G–C base pairs

Reprint requests to: Romina Oliva, Dipartimento di Scienze Applicate, Università di Napoli “Parthenope,” I-80133 Naples, Italy; e-mail: romina.oliva@uniparthenope.it; fax: +39 081 5522293.

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FIGURE 1. (A) A schematic representation of class I tRNA canonical structure. Nucleotides 15 and 48, forming the Levitt base pair, and other nucleotides involved in tertiary interactions are shown in squares. Nucleotides in the tRNA stems are represented by gray circles. Tertiary H-bonding interactions are indicated by dashed lines. (B) Ribbon representation of the yeast tRNA^{Phe} structure (PDB code: 1ehz; Shi and Moore 2000). Nucleotides G15 and C48 are shown as balls and sticks.

significantly reduces the psDNA duplex thermodynamic stability, both for base stacking and hydrogen bonding contributions (Rippe et al. 1990; Rentzeperis et al. 1992). Moreover, theoretical studies have shown that repulsive amino–amino and carbonyl–carbonyl contacts make the RWC arrangement unfavorable for an isolated G–C pair in the gas phase (Zhanpeisov et al. 1998).

In a previous paper we performed a systematic analysis of the geometry and stability of canonical tRNA tertiary interactions, focusing on the interbases' H-bonding contribution (Oliva et al. 2006). We purposely neglected other important forces, such as the base–backbone and backbone–backbone H-bonding interactions, the base–base stacking interactions, as well as RNA interaction with the environment. This strategy allowed us to gain the unambiguous result that six out of the nine canonical yeast tRNA^{Phe} tertiary interactions are held in place by H-bonds between the bases, i.e., that conformations with minimum energy are chosen for tRNA tertiary base pairing. Two of the misrepresented geometries concerned the third interacting base in the U8–A14–A21 and m2G10–C25–G45 base triplets. The only base pair whose optimized geometry did not correspond to the experimentally observed one is the G15–C48 Levitt pair (Oliva et al. 2006).

Instead of a RWC, the gas-phase optimized G15–C48 geometry is a bifurcated one, involving the central section of the G Watson–Crick face and the C keto group adjacent to the C1'. It is classifiable as a G–C Ww/Bs *trans*, according to the Leontis and Westhof nomenclature (Leontis and Westhof 2001) as extended by Lemieux and Major (2002) to account for bifurcated geometries. This

bifurcated base pair is highly nonisosteric with the G–C RWC base pair. Indeed, as compared to the RWC geometry, the G–C Ww/Bs *trans* base pair shows a different orientation of the glycosidic bonds, and a distance between the C1' atoms of G15 and C48 1.6 Å shorter. Consequently, it is not expected to be able to substitute the RWC geometry while preserving the RNA three-dimensional structure (Leontis et al. 2002; Lescoute et al. 2005).

On the other hand, all available tRNA experimental structures show the L-shaped conformation with the G15–C48 pair arranged in a RWC geometry. This strongly suggests that the G–C RWC geometry can be stabilized by additional effects. It is indeed well known from a number of cases that the biologically active form of tRNA, analogously to other macromolecules, is not necessarily fully imprinted in the molecule gene, and that it can depend upon the presence of metals, particularly magnesium (Lindahl et al. 1966; Adams et al. 1967; Ishida et al. 1971; Madore et al. 1999), and/or post-transcriptional chemical modifications (Agris 1996; Helm et al. 1998; Helm 2006).

To identify the possible factors stabilizing the G15–C48 Levitt tertiary pair in cytosolic tRNAs, we apply here a combined bioinformatics (sequence and structure analysis) and quantum mechanics approach. In particular, we explore (1) the effect of metal binding and (2) the effect of naturally occurring post-transcriptional chemical modifications. Interestingly, one of the few strong binding sites for divalent metal ions characterized for cytosolic tRNA molecules is localized on the G15 base (Jack et al. 1977; Hurd et al. 1979; Teeter et al. 1981; Shi and Moore 2000). The divalent metal ion is directly bound to the N7(G15) atom, which occupies one of the six positions of an octahedral coordination. In the crystallographic study reporting the best resolution structure now available for yeast tRNA^{Phe}, a Mg²⁺, Mn²⁺, or Co²⁺ ion is bound to the N7(G15) atom, depending upon crystallization conditions (Shi and Moore 2000). As far as the post-transcriptional chemical modifications are concerned, the analysis of available tRNA sequences shows that both positions 15 and 48 present only one post-transcriptional chemical modification. Modification at position 48 is a 5-methylcytosine (m5C), a neutral nucleotide bearing one additional methyl group on carbon 5. This type of modification is expected to have only a minor effect on the geometry and stability of tRNA tertiary interactions (Oliva et al. 2006). Interestingly, archaeal tRNAs present a complex post-transcriptional modification at position 15: 7-formamido-7-deazaguanosine (Gregson et al. 1993), abbreviated as fa7d7G (Sprinzl et al. 1998), adopted in the following, or G⁺ (Rozenki et al. 1999). Commonly known as archaeosine from its domain specificity, this is one of the most remarkable post-transcriptional chemical modifications identified in tRNA molecules. As a result of the G15 replacement by a 7-cyano-7-deazaguanine (preQ₀),

performed by Archaeal tRNA-guanine transglycosylase (ArcTGT) (Watanabe et al. 2001; Iwata-Reuyl 2003), and of subsequent enzymatic steps that remain to be clarified, archaeosine bears a charged imidino side chain on the C7 atom of the 7-deazaguanine core.

We have investigated the geometries and energies of the G15–C48 base pair, including the C1' ribose atoms, in the absence and in the presence of a hydrated Mg^{2+} cation bound to the N7 atom of G15 using accurate quantum mechanics methods. Analogous calculations have been performed for the 15–48 interaction when the post-transcriptional archaeosine modification is present. Moreover, since we are aware that gas-phase calculations may overemphasize electrostatic effects, in order to better approximate the polar tRNA environment we also performed the calculations in water using a continuum solvation model (Klamt and Schüürmann 1993). Finally, since stacking effects can be an important contribution to the base pair stability, we also investigated geometries and energies of the G15–C48 base pair when stacked between the U8–A14–A21 base triplet and the U59 base, as in yeast tRNA^{Phe}. The quantum mechanics approach adopted here has been validated by its successful application to the study of structure and energetics of H-bonded nucleic acid bases (Hobza and Šponer 1999; Fonseca Guerra and Bickelhaupt 2002; Šponer et al. 2004; Swart et al. 2004; Perez et al. 2005; Oliva et al. 2006), as well as of the effect of metal ions binding on several canonical and noncanonical base-pairing interactions (Burda et al. 1997; Šponer et al. 1999, 2001; Sychrovský et al. 2004). The continuum solvation model has been demonstrated to accurately reproduce hydration energies and H-bonding interactions of both neutral and charged systems (Klamt and Schüürmann 1993; Tomasi et al. 2005).

From our analysis, we can conclude that the presence of positive charges specifically located on the G15 base can stabilize the 15–48 RWC interaction and that metal binding and post-transcriptional archaeosine modification can both achieve the desired effect. Very interestingly, these two completely different but convergent strategies appear to be adopted in different life domains.

RESULTS

tRNA sequence analysis

We recorded all the possible nucleic acid base combinations occurring at positions 15–48 in the available cytoplasmic tRNA sequences (Sprinzl et al. 1998; Sprinzl and Vassilenko 2005). Seventy-seven percent of the combinations are of the G–C type, with or without post-transcriptional modifications. G15–C48 is the most frequent combination, occurring in 45% of the analyzed sequences. It is then followed by the G15–m5C48 combination, occurring in 26% of the sequences, by the A15–U48 combination, occurring in 17% of the sequences, and by the fa7d7G15–m5C48 combina-

tion, occurring in 5% of the sequences. The A15–C48 combination is present in 1% and other combinations, such as G15–G48, in <1% of the sequences.

When limiting the analysis to the 59 archaeal sequences (the available 161 sequences of archaeal tRNA genes were excluded because they lacked information about post-transcriptional modifications), the two archaeosine-containing combinations, fa7d7G15–m5C48 and fa7d7G15–C48, represent the first and third most frequent ones. They occur in 24 (41%) and 6 (10%) of the sequences, respectively, covering about one half of the cases. The G15–C48 pair is the second most populated combination observed in archaea, occurring in 21 (36%) of the available sequences. Archaeosine is the only modification occurring at position 15 in known tRNAs, and it seems not to depend upon the charged amino acid. To date archaeosine15 has been characterized in sequences from tRNAs for 15 different amino acids (Sprinzl et al. 1998; Sprinzl and Vassilenko 2005).

In the available sequences from bacteria and eukarya combinations different from the G–C type, such as A–U, A–C and G–G, represent ~20% of all interactions observed for the 15–48 pair while the G15–C48 pair (modified or unmodified) is found in all known archaeal sequences, with only two exceptions. This trend is confirmed when the set of available sequences of archaeal tRNA genes is analyzed. One hundred fifty-six out of 161 indeed have a G15–C48 pair.

Optimal geometries and interaction energies

Optimized geometries and relative interaction energies (ΔE) were computed for the G–C base pair in the RWC and Ww/Bs *trans* geometry, both in the gas phase and in water (Table 1). A G15–C48 pair is present in several tRNA molecules having their structures available. Among these, the yeast tRNA^{Phe} structure solved at 1.93 Å resolution (PDB code: 1ehz; Shi and Moore 2000) has been chosen as the reference system for our calculations.

The effect of Mg^{2+} binding on the G15 base has been simulated by optimizing both the G–C RWC and the Ww/Bs *trans* geometries in the presence of a hydrated Mg^{2+}

TABLE 1. Interaction energies in kilocalories/mole for the optimized interactions

System	Geometry	ΔE (Gas)	ΔE (Water)
G15–C48	RWC	— ^a	–8.4
G15–C48	Ww/Bs <i>trans</i>	–17.0	–7.5
fa7d7G15–C48	RWC	–28.0	–9.9
fa7d7G15–C48	Ww/Bs <i>trans</i>	–26.9	–8.1
(Mg[H ₂ O] ₅ G15–C48) ²⁺	RWC	–40.0	–11.4
(Mg[H ₂ O] ₅ G15–C48) ²⁺	Ww/Bs <i>trans</i>	— ^a	–9.4

^aUnstable.

cation bound to the N7(G15). The effect of the chemical modifications was also taken into account. Test calculations performed in the gas phase showed that the m5C modification has only minor effect on the G–C interaction (geometries virtually unchanged, ΔE affected by <4%). This is in perfect agreement with our previous results about the moderate effect of additionally methylated neutral nucleic acid bases on the geometry and stability of tRNA tertiary interactions (Oliva et al. 2006). Therefore, for the sake of simplicity, only the archaeosine modification is discussed here. We report optimized geometries and energies for the fa7d7G–C RWC and Ww/Bs *trans* geometries.

The G15–C48 base pair

As previously reported, gas-phase optimization of the X-ray G15–C48 base pair destroys the RWC geometry (Zhanpeisov et al. 1998; Oliva et al. 2006). We found that the optimized gas-phase G15–C48 pair presents the bifurcated H-bond pattern shown in Figure 2B and classifiable as a G–C Ww/Bs *trans* (Lemieux and Major 2002). As a consequence of the geometrical rearrangement from the RWC to the Ww/Bs *trans* structure the carbonyl–carbonyl and amino–amino repulsion, responsible for the instability of the isolated G–C RWC base pair (Zhanpeisov et al. 1998), are alleviated and the distance between the two C1' ribose atoms is reduced by ~ 2 Å. The gas-phase stability of the G–C Ww/Bs *trans*

geometry, $\Delta E = -17.0$ kcal/mol, is remarkably high. It is, in fact, intermediate between the calculated stability of the classical G–C and A–U WC base pairs (Šponer et al. 2004; Oliva et al. 2006).

To reproduce a more realistic polar environment we optimized the G–C pair in water starting both from the X-ray RWC geometry and from the gas-phase G–C Ww/Bs *trans* geometry. Differently from the gas phase optimization, in water optimization results in a stable RWC pair (see Fig. 3A for H-bond lengths). Interestingly, also the G–C Ww/Bs *trans* geometry is stable in water. The geometrical rearrangement, as compared to the calculated gas-phase geometry, is moderate (Figs. 2B, 3B). As expected, the absolute in water interaction energies for the RWC and the Ww/Bs *trans* geometry, $\Delta E = -8.4$ and -7.5 kcal/mol, respectively, are remarkably lower than in the gas phase. The difference in ΔE values in water (0.9 kcal/mol) indicates that the RWC geometry is moderately more stable than the Ww/Bs *trans* geometry.

Effect of base–base stacking

To investigate the role of stacking effects, we evaluated the relative energy of the Ww/Bs *trans* and RWC geometries for the G15–C48 base pair when stacked between the U8–A14–A21 triplet and the U59 base, as in yeast tRNA^{Phe}. In these calculations the U8–A14–A21 triplet and the U59 base were fixed to the X-ray structure. Coordinates of the G15 and C48 C1' atoms were also fixed to the X-ray values in the case of the RWC geometry and to values as near as possible to the X-ray values in the case of the Ww/Bs *trans* geometry (as obtained by best superimposition of the G15 and C48 C1' atoms on the corresponding ones in the 1ehz X-ray structure). The rest of the G15–C48 base pair was fully relaxed, and the optimized geometries are shown in Figure 4.

Differently from the in water results, the Ww/Bs *trans* G15–C48 base pair is found to be more stable than the RWC G15–C48 base pair by 1.4 kcal/mol, when stacked between the U8–A14–A21 triplet and the U59 base. Although the size of the systems forced us to use a less thoroughly tested approach (see Computational Details), our results indicate that the G15–C48 base pair prefers a bifurcated geometry even in the presence of stacking effects.

Effect of metal binding

In the gas phase, the $(\text{Mg}[\text{H}_2\text{O}]_5\text{G15–C48})^{2+}$ complex shows an opposite behavior as compared to an isolated G–C pair, i.e., the RWC geometry is stable, whereas the Ww/Bs *trans* geometry does not correspond to an energy minimum and converges into the RWC geometry; see Figure 2C,D. The gas-phase RWC optimized geometry of the $(\text{Mg}[\text{H}_2\text{O}]_5\text{G15–C48})^{2+}$ complex is quite similar to the X-ray one (see Figs. 2C, 3G). The gas-phase interaction

FIGURE 2. RWC and Ww/Bs *trans* optimized geometries in the gas phase for the G–C pair (A,B), for the $[\text{Mg}(\text{H}_2\text{O})_5\text{G15–C48}]^{2+}$ complex (C,D), and for the fa7d7G–C pair (E,F) (fa7d7G is the abbreviation for archaeosine). H-bond distances between the heavy atoms are reported in angstroms.

FIGURE 3. (A–F) RWC and Ww/Bs *trans* optimized geometries in water. (G,H) Examples of G–C RWC and Ww/Bs *trans* geometries in X-ray RNA structures, including the ribose–phosphate backbone; relative PDB codes are also reported. H-bond distances between the heavy atoms are reported in angstroms.

energy of the RWC geometry is remarkably high, $\Delta E = -40.0$ kcal/mol. This is reasonable considering that the double positive charge on the $(\text{Mg}[\text{H}_2\text{O}]_5\text{G15})^{2+}$ fragment is not damped by solvent effects, and it is in line with the gas-phase interaction energy of H-bonded base pairs when a net charge is localized on the base pair (Šponer et al. 2004; Oliva et al. 2006).

In water optimization of the $(\text{Mg}[\text{H}_2\text{O}]_5\text{G15–C48})^{2+}$ complex results in stable RWC and Ww/Bs *trans* geometries (Fig. 3C,D), similarly to what is found for the uncomplexed G–C base pair. Absolute interaction energies are considerably reduced in water; ΔE is -11.4 and -9.4 kcal/mol for the RWC and the Ww/Bs *trans* geometry, respectively. Thus, in water and in the presence of the metal, the RWC base pair is more stable than the Ww/Bs *trans* pair by 2.0 kcal/mol.

As a net result, the presence of the hydrated Mg^{2+} cation bound to N7(G) shifts the energy minimum for the parallel G–C pair from the Ww/Bs *trans* to the RWC geometry in the gas phase, and stabilizes the G–C RWC base pair by 3 kcal/mol in water (see Table 1).

Finally, we also evaluated the interaction energy between the hydrated Mg^{2+} cation and the isolated G base, as well as

between the hydrated Mg^{2+} cation and the G15–C48 RWC base pair. In this case, we considered the following two reactions:

where the additional water molecule has been included to preserve a hexacoordinated Mg^{2+} cation. In both reactions 1 and 2 the right side is preferred, by 12.1 and 15.2 kcal/mol, respectively. This implies that coordination of the Mg^{2+} cation to the N7(G) atom is favored and that formation of the base pair further favors Mg^{2+} binding by ~ 3 kcal/mol.

Effect of chemical modification

Gas-phase optimization shows that the RWC geometry is stable for the fa7d7G–C base pair, differently from the simple G–C base pair (see Fig. 2A,E). The gas-phase Ww/Bs *trans* geometry is also stable and rather similar to that calculated for the G–C base pair (Fig. 2B,F). The gas-phase interaction energy of the RWC and of the Ww/Bs *trans* geometries, $\Delta E = -28.0$ and -26.9 kcal/mol, respectively, is intermediate between the gas-phase ΔE of the G–C and $[\text{Mg}(\text{H}_2\text{O})_5\text{G–C}]^{2+}$ systems.

In water, optimization of the RWC and Ww/Bs *trans* fa7d7G–C base pairs results in geometries quite similar to those calculated in the gas phase. Only minor differences in the H-bond lengths are observed (Fig. 3E,F). Again, absolute interaction energies are considerably reduced in water, $\Delta E = -9.9$ and -8.1 kcal/mol for the RWC and the Ww/Bs *trans* geometry, respectively. Thus, for the fa7d7G–C base pair the RWC geometry is more stable than the Ww/Bs *trans* one both in the gas phase and in water, by 1.1 and 1.8 kcal/mol, respectively (i.e., the relative stability of the RWC geometry is further increased by 0.7 kcal/mol when going from the gas phase to water).

FIGURE 4. Optimized geometries of the G–C RWC and G–C Ww/Bs *trans* base pairs, when stacked between the U8–A14–A21 triplet and the U59 base fixed to the coordinates of the yeast tRNA^{Phe} structure (PDB code: 1ehz). H-bond distances between the G15–C48 heavy atoms are reported in Ångströms.

As a net result, the fa7d7G–C RWC geometry is stable in the gas phase and is stabilized by 1.5 kcal/mol in water as compared to the unmodified G–C interaction (see Table 1).

Electron density analysis

To shed light on the increased stability of the RWC geometry in the presence of the hydrated Mg^{2+} cation and of the archaeosine modification, we compared the electron density of the different systems. Figure 5 reports the differences of electron density in water between $(\text{Mg}[\text{H}_2\text{O}]_5\text{G})^{2+}$ and fa7d7G and a simple G base (see the Computational details section).

Inspection of Figure 5 clearly indicates that the presence of the hydrated Mg^{2+} cation substantially reduces electron density around all the H atoms, including those on N1 and N2, involved in H-bonds with C48 in both analyzed geometries. This explains the increased stability of both the G–C RWC and Ww/Bs *trans* geometries in the presence of the hydrated Mg^{2+} cation. Additionally, the reduced density around the H atoms of the N2 amino group will result in reduced repulsion with the amino group of the C base in the RWC geometry. At the same time, the strong interaction between the hydrated Mg^{2+} cation and the O6 atom polarizes the electron density toward the cation, at the expense of electron density in proximity of the N1 atom (Fig. 5A). This results in reduced repulsion with the carbonyl group of the C base in the RWC geometry. Reduction of both the amino–amino and carbonyl–carbonyl repulsive interactions, due to the hydrated Mg^{2+} cation, further favors the G–C RWC geometry, which is stabilized by two strong H-bonds (one donor–one acceptor).

The effect of the 7-formamidine group on the 7-deazaguanine core is very similar to that of the hydrated

Mg^{2+} on the G base. Although slightly weaker in the fa7d7G system, as compared to the $(\text{Mg}[\text{H}_2\text{O}]_5\text{G})^{2+}$ system, both reduction of electron density around the N1 and N2 protons and polarization of the electron density around O6 away from the N1 atom are observed (Fig. 5B). (Note that for fa7d7G a G-like numbering scheme has been adopted here to simplify the comparison, instead of the originally proposed scheme) (Gregson et al. 1993). The fa7d7G modification can thus be viewed as an effective mimic of a hydrated Mg^{2+} ion bound to the N7(G) atom.

GC RWC and Ww/Bs *trans* base pairs in RNA structures

In light of the comparable stability demonstrated in water by the G–C Ww/Bs *trans* and RWC base pairs, we searched for the occurrence of such geometries in known RNA structures, solved at a resolution of 3 Å or better (updated to November 30, 2006).

As expected, G–C RWC base pairs were found at positions 15–48 in tRNAs (41 structures for 14 tRNA molecules differing by source and/or specificity, i.e., charged amino acid). Four G–C RWC base pairs were also found in 32 structures for *Haloarcula marismortui* 23S rRNA and six G–C RWC base pairs in two structures for *Thermus thermophilus* 23S rRNA. (A complete list of the PDB codes and details about the residues involved in the interactions and relative H-bond lengths can be found in the Supplemental Tables S1 and S2 at http://www.dsa.uniparthenope.it/dsa/Portals/8/Personale/Romina.Oliva/supplementary_Oliva_et_al_Rna07.pdf).

Three occurrences of the G–C Ww/Bs *trans* base pair were found for three molecules in five PDB files: a 20-mer (PDB code: 1ec6), a 40-mer RNA (PDB codes: 1nta/1ntb), and 23S rRNA from *T. thermophilus* (PDB codes: 2j01/2j03), at positions 10–7 (chain C), 12 (chain A)–109 (chain B) and 250–201 (chain A, with nt C201 also involved in a bent WC base pair with nt G194), respectively. They are not included in the Non Canonical Base Pair Database (NCIR; Nagaswamy et al. 2002), and were not listed by Lemieux and Major in their repertoire of occurring noncanonical base pair interactions, updated to February 2001 (Lemieux and Major 2002). Note that the 1nta, 1ntb, 2j01, and 2j03 structures were released after this latter analysis was performed.

The G–C RWC base pair from the best resolution tRNA structure (PDB code: 1ehz) and the G–C Ww/Bs *trans* base pair from the 1ntb structure are reported as examples in Figure 3G,H.

DISCUSSION

We have previously demonstrated that tRNA prefers solutions of minimum energy for its tertiary base pairing and that the G15–C48 Levitt pair seems to represent an exception since in the gas phase a stable Ww/Bs *trans*

FIGURE 5. Electron density difference, in the base plane, between $(\text{Mg}[\text{H}_2\text{O}]_5\text{G})^{2+}$ and the simple G base (A), and archaeosine (fa7d7G) and the G base (B). Density difference curves are plotted between -0.02 and 0.02 atomic units (a.u.), with a spacing of 0.001 a.u. Dashed (full) lines refer to negative (positive) density difference curves, i.e., to areas where the Mg^{2+} -bound or the archaeosine base presents reduced (increased) electron density as compared to the simple G base.

bifurcated geometry is reached starting from the experimental RWC geometry (Oliva et al. 2006). We show here that both the geometries are stable in water, but the difference in stability, 0.9 kcal/mol, is still quite small. Moreover, our results suggest that base–base stacking contributions in the context of the yeast tRNA^{Phe} structure should favor the bifurcated geometry. Note that the stability of the G–C Ww/Bs *trans* geometry is demonstrated here not only by accurate quantum mechanics calculations, but also by its identification in known RNA structures.

We also investigated the effect of metal binding and of chemical modification on the G15 base. Metals, and particularly magnesium, are known to be required for a functionally active conformation to be acquired in many tRNAs (Lindahl et al. 1966; Adams et al. 1967; Ishida et al. 1971; Madore et al. 1999). It has also been shown that divalent metal ions are particularly important in maintaining the yeast tRNA^{Phe} tertiary core structure (Friederich et al. 1998). Interestingly, one of the few strong metal binding sites characterized for cytosolic tRNAs is localized on G15 (Jack et al. 1977; Hurd et al. 1979; Teeter et al. 1981; Shi and Moore 2000). We have shown here that the binding of a hydrated Mg²⁺ ion to N7(G15) induces an electron density redistribution on the nucleic acid base (Fig. 5), which results in an efficient stabilization of the RWC geometry, required for the G15–C48 “correct” tertiary interaction, over the easily accessible alternative Ww/Bs *trans* bifurcated geometry. Our in-water calculations show that, in the presence of the metal coordinated to N7(G15), the RWC geometry is stabilized by 3.0 kcal/mol, while the energy gap between the two geometries raises to 2.0 kcal/mol (Fig. 6).

The structural core of the L-shaped tRNA structure also represents one of the two sites (the other being the anticodon domain) where most tRNA nucleotide modifications occur. A body of data suggests a primarily structural role for such modifications. Apart from rare cases where single modifications prevent alternative tRNA folding by impairing additional secondary base pairing, the core modifications are shown to provide native tRNAs with

the advantage of lower conformational flexibility, improved thermal stability and improved aminoacylation parameters (Helm 2006). Very interestingly, half of known archaeal tRNA sequences, for which information on modification is available, have an archaeosine at position 15. Such a complex modification presents an imidino side chain on the C7 atom of a 7-deazaguanine core, thus precluding the possibility of metal binding to the N7 atom. Location of archaeosine at such a position of tRNA, involved in the crucial 15–48 tertiary interaction, has led to the speculation that it might play a role in stabilizing the tRNA tertiary structure. Quite recently the mode of binding of *Pyrococcus horikoshii* ArcTGT to tRNA^{Val} has been elucidated by the determination of the structure of the complex, solved at 3.3 Å resolution (Ishitani et al. 2003). In the complex, the tRNA is recognized by the enzyme in an alternative “λ-shaped” conformation, where the 15–48 as well as most of the tertiary interactions are disrupted. This indirectly confirms the instability of the archaeal tRNA functional conformation in the absence of the archaeosine modification.

The putative stabilizing effect of archaeosine has been till now ascribed to generic electrostatic effects, such as interactions of the positively charged formamidine group with close in space 5′-phosphate groups (Iwata-Reuyl 2003). Here we demonstrate that the structural role of archaeosine, analogously to a metal bound to N7(G15), could be primarily that of stabilizing the correct 15–48 base pairing interaction. Our in-water calculations indicate that, in the presence of the archaeosine modification, the RWC geometry is stabilized by 1.5 kcal/mol, while the energy gap between the RWC and Ww/Bs *trans* geometries doubles from 0.9 to 1.8 kcal/mol (Fig. 6). The presence of a positively charged formamidine group on the C7 atom thus works as a “pure covalent mimic” of the coordinated metal. This is clearly illustrated by the similarity in the electron density redistribution induced on the G base by metal binding and chemical modification (Fig. 5).

Metal binding and post-transcriptional modification have been long established to play an interchangeable stabilizing role on the tRNA structure (Kopper et al. 1983; Hyde and Reid 1985; Agris et al. 1986; Hall et al. 1989; Agris 1996). Many studies reported how the lack of modification in tRNA in vitro transcripts can be compensated by an increased concentration of Mg²⁺ ions to obtain functional structures (Sampson and Uhlenbeck 1988; Perret et al. 1990; Maglott et al. 1998; Serebrov et al. 1998). We illustrate here the interchangeable stabilizing role of metal binding and post-transcriptional modification on a specific tRNA tertiary interaction, the G15–C48 Levitt base pair, provided with a physicochemical explanation of the phenomenon.

The convergence of two completely different strategies toward the stabilization of a crucial interaction in tRNAs that we discuss here is, in our view, a fascinating example of

FIGURE 6. Energy trend of the G–C RWC and Ww/Bs *trans* geometries for the three analyzed systems, in water and in the gas phase.

the ingenuity of evolutionary mechanisms. The different route taken by archaea can be due to an evolutionary accident, but it can also reflect difference in selective constraints, maybe related to the extreme environments where most of these organisms live. The binding energy of Mg^{2+} to the G15 base (12–15 kcal/mol) is far closer to that of a H-bond interaction than to that of a carbon–carbon σ -bond. Thus, differently from the chemical fa7d7G modification, metal coordination to the G15–C48 base pair should be considered reversible, especially at high temperatures, when kinetic energy increases and the entropic contribution becomes important. Notably, in archaea, which live in more extreme environmental conditions, an enzymatic post-transcriptional modification seems to be preferred over the possibly more environment-dependent metal binding.

Finally, our results indicate that, also in the presence of the Mg^{2+} cation or of the archaeosine modification, the energy difference between the G–C RWC and Ww/Bs *trans* geometries is moderate. Considering that large conformational changes are documented in the tRNA function (Valle et al. 2002, 2003; Frank et al. 2005), it might be speculated that the presence of two accessible geometries of the Levitt base pair, as we have discussed here, may assist the structural adaptations of tRNA during its movement through the ribosome.

MATERIALS AND METHODS

Sequence and structure analysis

The set of analyzed tRNA sequences corresponds to the 466 cytosolic and chloroplastic tRNA sequences and 1732 tRNA genes from the Sprinzl database (Sprinzl et al. 1998; Sprinzl and Vassilenko 2005). The subset of PDB structures used in this work, updated to November 30, 2006, is composed of the 519 structures containing RNA molecules solved at a resolution of 3.0 Å or better. To find occurrences of G–C base pairs in the RWC geometry, we searched the data set for G and C nucleotides presenting the N1(G)–O2(C) and N2(G)–N3(C) H-bonds (threshold 3.4 Å) and not presenting the *cis* WC O6(G)–N4(C) H-bond (threshold 4.0 Å). To avoid G–C *cis* WC geometries, we also set a limit on the C1'(G)–C1'(C) atoms' distance (7.0 Å). To find occurrences of G–C base pairs in the Ww/Bs *trans* arrangement, we searched the data set for G and C nucleotides presenting the N1(G)–O2(C) and N2(G)–O2(C) H-bonds (threshold 3.4 Å) and not presenting the *cis* WC O6(G)–N4(C) H-bond (threshold 4.0 Å). All the hits were visually checked in order to exclude false positives. The complete list of true positives is available as Supplemental information at http://www.dsa.uniparthenope.it/dsa/Portals/8/Personale/Romina.Oliva/supplementary_Oliva_et_al_Rna07.pdf.

Computational details

The TURBOMOLE package (Ahlrichs et al. 1989) was used for all the calculations reported here. A density functional theory (DFT)

approach based on the hybrid B3LYP functional (Lee et al. 1988; Becke 1993, 1996) and the cc-pVTZ basis set (Dunning 1989) was used for all geometry optimizations. Interaction energies were calculated on the B3LYP/cc-pVTZ geometries at the second-order Møller–Plesset (MP2) level of theory (Møller and Plesset 1934) in the framework of the resolution of identity approximation (Weigend and Häser 1997) and using the more extended aug-cc-pVTZ basis sets (Dunning 1989). All interaction energies were corrected for basis set superimposition error (BSSE) using the counterpoise procedure (Boys and Bernardi 1970).

The total interaction energy ΔE was calculated as

$$\Delta E = E_{XY} - (E_X^0 - E_Y^0) - \text{BSSE} \quad (3)$$

where E_{XY} is the energy of the optimized X–Y base pair, E_X^0 and E_Y^0 are the energies of the isolated and optimized X and Y bases, and BSSE is the basis set superimposition error. This is the standard approach in this kind of calculation (Burda et al. 1997; Hobza and Šponer 1999, 2002; Šponer et al. 2004; Oliva et al. 2006). Coordinates and energies of the calculated structures can be found at http://www.dsa.uniparthenope.it/dsa/Portals/8/Personale/Romina.Oliva/supplementary_Oliva_et_al_Rna07.pdf.

When stacking effects on the G15–C48 base pair stability were considered, the approach developed by Grimme (2004) to investigate base–base stacking was adopted. In fact, standard DFT usually fails to correctly reproduce Van der Waals forces (Kristyán and Pulay 1994; Pérez-Jordá and Becke 1995), which are at the basis of stacking interactions, and MP2 calculations on systems composed by six bases are still unfeasible. Grimme's (2004) approach consists of the addition to standard DFT of an empirical correction term describing Van der Waals forces. The PBE functional (Perdew et al. 1996) with the SVP basis set (Weigend and Ahlrichs 2005) and a classical R^{-6} term based on Miller's (1990) atomic parameters were used to describe the DFT and the Van der Waals terms, respectively.

Electron density analysis

Comparative analysis of the electron density of a single G base with that of the $[\text{Mg}(\text{H}_2\text{O})_5\text{G}]^{2+}$ complex, as well as with that of the fa7d7G base, was performed as follows. First, the geometry of the $[\text{Mg}(\text{H}_2\text{O})_5\text{G}]^{2+}$ complex and of the fa7d7G base was optimized at the B3LYP/cc-pVTZ level of theory. C_s symmetry was imposed to the systems, and electron density analysis was performed in the symmetry plane (coincident with the purine plane). After optimization, we compared the RIMP2/aug-cc-pVTZ electron densities of the $[\text{Mg}(\text{H}_2\text{O})_5\text{G}]^{2+}$ complex, ρ^{Mg} , and of the G base with the geometry it has in the $[\text{Mg}(\text{H}_2\text{O})_5\text{G}]^{2+}$ complex, $\rho^{\text{G/Mg}}$. The plotted electron density difference is $\rho^{\text{Mg-G/Mg}} = \rho^{\text{Mg}} - \rho^{\text{G/Mg}}$. A similar procedure was followed for the fa7d7G base. In this case, we compared the RIMP2/aug-cc-pVTZ electron densities of fa7d7G, ρ^{fa7d7G} , and of the G base obtained by removing the formamidine group from the optimized fa7d7G base, and replacing the C7 atom of fa7d7G with a N atom, $\rho^{\text{G/fa7d7G}}$. The plotted electron density difference is $\rho^{\text{fa7d7G-G/fa7d7G}} = \rho^{\text{fa7d7G}} - \rho^{\text{G/fa7d7G}}$. We adopted this procedure to have perfect overlap between the skeletons of the G and fa7d7G bases, and, consequently, meaningful electron density differences.

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Mg²⁺ binding and archaeosine modification stabilize the G15–C48 Levitt base pair in tRNAs

Romina Oliva, Anna Tramontano and Luigi Cavallo

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