Chapter I

Introduction
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Transfer RNA is primarily originated in the pre-biotic RNA world as a functional minihelices (Buechter and Schimmel 1993). Basically transfer RNA plays crucial role in the protein biosynthesis process as an adaptor molecule (Crick 1955) along with this it also play an important role in the various metabolic processes of the cell such as cell wall biosynthesis, heme biosynthesis, antibiotics synthesis as well as in the modification of bacterial membrane lipids etc. (Francklyn and Minajigi 2010). Transfer RNA was discovered by Hoagland and Zamecnik almost half a century ago in 1957 (Hoagland et al 1958). The primary sequence (cloverleaf model) of yeast tRNA alanine was first discovered by Robert Holley and his coworkers (Holley et al 1965). Transfer RNA contains nearly 73-93 nucleotides. It has an acceptor stem, D stem-loop, anticodon stem-loop and TΨC stem-loop. Along with this tRNA bears an extra variable loop between TΨC and anticodon stem. Variable loop comprises of about 3 to 21 nucleotides, which play key role in stabilizing the tertiary structure of transfer RNA (Rich and RajBhandary 1976).

Immediately after the discovery of transfer RNA it was found that it contains post-transcriptionally modified nucleosides (Dunn 1959). These modified nucleosides are the derivatives of four common nucleosides such as adenosine, uracil, guanosine and cytidine (Bjork et al 1992). All the characterized tRNA species bears numerous modifications in base or in ribose sugar moiety. The tRNA modifications involve simple methylation of base and of their ribose sugar along with this it also shows number of complex chemical modifications due to acetylation, thiolation, hydrogenation and isomerization of four common nucleosides called as hypermodified nucleosides (Limbach et al 1994; Sprinzl et al 1996; 1998). Many of these modifications are conserved within various domains of life such as archaea, bacteria, and eukaryotes suggesting their important structural as well as functional role (Juhling et al 2009). These modifications are species-dependent and/or sequence-specific e.g. tRNA\textsuperscript{Phe} from Mycoplasma, E. coli, and from various plants contain m\textsuperscript{1}G, mS\textsuperscript{2}i\textsuperscript{6}A, and O\textsuperscript{2}yW at 3\textsuperscript{'} adjacent 37\textsuperscript{th} position (Nishimura 1972; Cedergren et al 1981). The analysis of 561 sequenced tRNAs gene from different organisms have been performed (Sprinzl and Vassilenko 2005). This study showed that near about 8 modifications are found per tRNA species (Phizicky and Alfonzo 2010). Near about 107
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modifications have been found in RNA out of that about 92 modifications are from transfer RNA (Czerwoniec et al 2009; Sprinzl et al 1998; Rozenski et al 1999; Grosjean 2005). The modified nucleosides are most frequently occur in the anticodon loop of tRNA, particularly at ‘wobble’ 34 and 3’ adjacent 37 position whereas other common modifications such as m1A, m6A, Cm, m2G, m2G, Dihydrouridine and Ribothymidine occur at other part of tRNA (Sprinzl et al 1998, Juhling et al 2009). It has been postulated that hypermodifications occur at 34 and 37 positions does not affect or change the biological properties of transfer RNA but rather serves for the fine-tuning of smooth and in-phase protein biosynthesis process by adjusting the codon-anticodon association energy (Grosjean and Chantrenne 1980). The modifications those occur in other parts of tRNA except anticodon loop are essential to maintain the structural stability of tertiary structure of tRNA molecule (Davanloo et al 1979; Steinberg and Cedergren 1995; Bavi et al 2011).

Extensively modified nucleosides have been found at 3' adjacent (37) position in the anticodon loop of tRNA which play crucial role in restricting the extended Watson-Crick base pairing sites and provide open ordered anticodon loop conformation for codon binding (Vendeix et al 2012). Transfer RNA gene sequence database analysis study shows that adenosine is found at 3' adjacent 37th position in 80% and guanosine in 20% of tRNAs (Sprinzl et al 1998). The hydrophobic base modifications such as t6Ade, mS2t6Ade, io6Ade, mS2io6Ade are present at 37 position when adenosine is present at 36 position in the anticodon loop of tRNA whereas, uridine at 36 position has hydrophilic base modifications such as t6Ade, mS2t6Ade, m6t6Ade (Motorin and Grosjean 2001; Bjork et al 1987; Chheda et al 1969; Schweizer et al 1969; 1970).

In the present study we have investigated the structural significance of hypermodified nucleosides 3-hydroxynorvalylcarbamoyl Adenosine, hn6Ade and 2-methylthio derivative mS2hn6Ade present at 3'-adjacent (37th) position in the anticodon loop of hyperthermophilic bacteria and archaia as well as from the psychrotolerant archaia (Reddy et al 1992; Noon et al 2003). The hypermodified nucleosides hn6Ade and mS2hn6Ade are the derivatives of N(6)-threonylcarbamoyl Adenosine, t6Ade by the addition of methyl (-CH3) and 2-methylthio (-SCH3) group (Reddy et al 1992). The hydrophilic class of hypermodified nucleoside t6Ade and its relative derivatives such as m6t6Ade, mS2t6Ade,
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hn°Ade and mS²hn°Ade generally reads ANN codon (N being one of the four usual nucleotides A, G, U, and C) during protein biosynthesis process (Reddy et al 1992). The X-ray crystallographic investigations on the structure of t°Ade showed that the ‘distal’ orientation of the N(6)-substituted threonylcarbamoyl side chain forms hydrogen bond between N(1) and HN(11) (Parthasarathy et al 1977). Similarly, the potassium and rubidium salts of N-(purin-6-ylcarbamoyl)-L-threonine, t°Ade also preferred the ‘distal’ orientation. Hence, the distal orientation of the N(6)-substituted side chain and the interaction between N(1) of adenosine and HN(11) of amino acid blocks the two sites N(6)-H and N(1) of adenine that are used for complementary base pairing (Parthasarathy et al 1974; Adamiak et al 1975) and may be important for enhancing the single stranded conformation of anticodon loop of tRNA and restrict the extended codon-anticodon interaction at 3’-adjacent site of tRNA (Parthasarathy et al 1977; 1974).

The previous conformational studies using quantum chemical semi-empirical PCILO method have shown the ‘distal’ orientation and hydrogen bonding interaction between N(1) and HN(11) for the hypermodified nucleic acid bases such as t°Ade, m°tn°Ade, and mS²t°Ade (Tewari 1987; 1990). The post-transcriptional hypermodified nucleosides t°Ade, m°tn°Ade, mS²t°Ade, hn°Ade and mS²hn°Ade are present at 3’-adjacent position of tRNA and respond to codon beginning with adenosine (Morin et al 1998; Ishikura et al 1969; Powers and Peterkofsky 1972; Reddy et al 1992). The 3-hydroxynorvalylcarbamoyl adenosine, hn°Ade and 2-methylthio derivative, mS²hn°Ade contains a 3-hydroxynorvalyl substituent, which is not a common alpha-amino acid. Hence it is of great interest to find out the structural significance of hypermodified nucleosides containing hydrophilic amino acid side chains.

The ‘wobble’ 34th position base modifications play crucial role in codon recognition by restricting or enlarging the scope of wobble base pairing (Yokoyama et al 1995). The ‘wobble’ 34 position cytosine base modifications such as k²C (Harada and Nishimura 1974), f⁸C (Takemoto et al 2009) and ac⁴C (Stern and Schulman 1978) reads more than one codons (i.e. AUG for Methionine and AUA for Isoleucine). The modified nucleoside N(4)-acetylcystidine, ac⁴C occur at ‘wobble’ (34) position in anticodon loop of certain archaeal and bacterial tRNAs (Stern and Schulman 1978) and in case of eukaryotes it has been observed at 12 position in D stem region (Sprinzl and Vassilenko 2005). The ac⁴C
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Modification has also been occur in 5S rRNA of Pyrodictum occultum (Bruenger et al 1993), 18s rRNA of Dictyostelium discoideum (McCarroll et al 1983) and from rat liver cells (Thomas et al 1978). The modified base N(4)-acetylycytidine at 34 position reads AUG for Methionine codon and prevent misreading of AUA for Isoleucine codon during protein biosynthesis process (Stern and Schulman 1978). Basically the gene tmcA is responsible for the formation of ac^4C at ‘wobble’ (34) position in anticodon loop of tRNA^Met in Escherichia coli (Ikeuchi et al 2008), whereas in case of Saccharomyces cerevisiae, Tan1 protein was identified for the synthesis of ac^4C at 12 position in tRNA^Ser with unknown partner enzyme (Johansson and Bystrom 2004; Silva et al 2008). The lack of Tan1 protein, required for formation of ac^4C at position 12 in yeast tRNA^Ser and tRNA^Leu have a growth defects (Kotelwala et al 2008). The NMR study showed that N(4)-acetylation stabilizes the C3’ endo form of ribose ring conformation of tRNA at higher temperature (Kawai et al 1992). The crystal structure study of ac^4C shows proximal orientation of N(4)-substituted acetyl group which is important for the correct recognition of mRNA codons (i.e. AUG/AUA) during protein biosynthesis process (Parthasarathy et al 1978).

The structural significance of 3’-adjacent and ‘wobble’ (34) position hypermodified nucleosides was studied at atomic level by using the conformational energy calculation (Tewari 1987; 1996; Sonawane 2008, 2000) and using molecular dynamics (MD) simulation method (Sonavane et al 2002; Kumbhar and Sonawane 2011b; Kumbhar et al 2012b). The previous studies have been carried out on the conformational preferences of hypermodified nucleic acid base present at 37th position in the anticodon loop of tRNA such as i^6Ade, mS^2i^6Ade (Tewari 1988), io^6Ade, Cis-io^6Ade, Trans-io^6Ade, Cis-mS^2-io^6Ade, Trans-mS^2-io^6Ade (Sonawane et al 2002), t^6Ade (Tewari 1987), m^6t^6Ade and mS^2t^6Ade (Tewari 1990), yW and O^3hyW (Kumbhar and Sonawane 2011b, Kumbhar et al 2012b), m^2G and m^2t^2G which occur at the hinge region (Bavi et al 2011) and the protonation effects of g^6Ade (Tewari 1994; 1995; 1996; Sonawane et al 2000). Similar efforts have been made on the 34th position hypermodified nucleosides queuosine (Sonavane et al 2002) and k^2C (Sonawane and Tewari 2008; Sonawane et al 2011; Sambhare et al 2011) using PCILO method.

Modified nucleic acid base have some physiological effects (Saran and Ojha 1991), such as the nucleoside antibiotic, 3’-azido-3’-deoxythymidine (AZT) or azidothymidine has
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been shown great promise in inhibiting the Human Immuno Deficiency Virus (HIV) and in reducing mortality among AIDS patients. The conformational preferences of this modified antibiotics has studied using semi-empirical quantum chemical PCIILO method and has important biological significance in explaining the drug action of azidothymidine (Saran and Ojha 1991). Similarly, the conformation of nucleoside antibiotics virazole, tubercidin, conformycin (Saran and Mitra 1979) and peptide nucleic acid (PNA) monomer (Sharma et al 2009) have also been studied at molecular level. The quantum chemical semi-empirical PM3 method has been used to understand the conformational behavior of modified nucleosides yW and O²hyW (Kumbhar and Sonawane 2011b, Kumbhar et al 2012b) as well as various biologically active molecules (Ferreira et al 2012; Labidi 2012; Anisimov and Cavasotto 2011).

The present study has been performed to find out the structural significance of post-transcriptionally modified nucleosides 3-hydroxynorvalylcarbamoyl Adenosine, hn⁶Ade and its 2-methylthio derivative, mS²hn⁶Ade occur at 3'-adjacent (3⁷th) position and N(4)-acetylcytidine, ac⁴C present at ‘wobble’ 3⁴th position along with t⁶Ade at 3⁷th position in the anticodon loop of transfer RNA. Here, we performed the conformational energy calculations using quantum chemical semi-empirical PCIILO and RM1 methods along with geometry optimizations to compare the salient features of preferred most stable conformation of the hypermodified nucleosides. Geometry optimizations have been performed using quantum chemical semi-empirical, molecular mechanics, HF and Density Functional Theory (DFT) methods.

The molecular dynamics simulations have also been made to see the hydration effects on the preferred most stable conformation of modified nucleic acid bases ac⁴C, hn⁶Ade and, mS²hn⁶Ade at isolated level as well as when present in the model anticodon stem loop (ASL) segment of tRNA. Conformational energy calculation and molecular dynamics simulation studies have been used to find out the structural role of hypermodified nucleosides hn⁶Ade, mS²hn⁶Ade, ac⁴C and t⁶Ade at isolated, diphosphate, trinucleotide, and in the anticodon loop segments as well as anticodon stem-loop (ASL) segment of transfer RNA.
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Hence, this computational biochemistry study could be useful to understand the structural significance of modified nucleosides such as 3-hydroxynorvalylcarbamoyl Adenosine, $hn^6Ade$ and its 2-methylthio derivative, $mS^2hn^6Ade$, N(4)-acetylcystidine, $ac^4C$, and N(6)-threonylcarbamoyl Adenosine, $t^6Ade$ present in the anticodon loop of tRNA. It could also be helpful to find out the effect of these modified nucleosides on the anticodon loop structure as well as on codon-anticodon interactions during protein biosynthesis process.