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THE ROLE OF INITIATOR tRNA\textsubscript{met} IN FIDELITY OF INITIATION OF PROTEIN SYNTHESIS

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The proper arrangement of amino acids in a protein determines its proper function, which is vital for the cellular metabolism. This indicates that the process of peptide bond formation requires high fidelity. One of the most important processes for this fidelity is kinetic proofreading. As biochemical experiments suggest that kinetic proofreading plays a major role in ensuring the fidelity of protein synthesis, it is not certain whether or not a misacylated tRNA would be corrected by kinetic proofreading during the peptide bond formation. Using 2-layered ONIOM (QM/MM) computational calculations, we studied the behavior of misacylated tRNAs and compared the results with these for cognate aminoacyl-tRNAs during the process of peptide bond formation to investigate the effect of nonnative amino acids on tRNAs. The difference between the behavior of initiator tRNA\textsubscript{met} compared to the one for the elongator tRNAs indicates that only the initiator tRNA\textsubscript{met} specifies the amino acid side chain.

Keywords
ONIOM; peptide bond formation; tRNA; ribosome

INTRODUCTION

Proteinsynthesis proceeds by transfer of the growing polypeptide chain from the peptidyl-tRNA bound to the ribosomal P-site to the incoming aminoacyl-tRNA in the adjacent A-site. After the translocation of the nascent peptidyl-tRNA from A-site to the P-site and the misacylated tRNA from the P-site to the E-site of the ribosome by the action of elongation factor G, the next codon in the empty A-site becomes exposed to the new aminoacyl-tRNA
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The tRNA binding sites are located at the interface between the ribosomal subunits, with the decoding center (DC) on the small subunit\textsuperscript{[2]} and the peptidyl transfers center (PTC) on the large subunit.\textsuperscript{[3]} Synthetic polynucleotide containing AUG and/or GUG codons as well as natural mRNA have been used extensively in order to elucidate the mechanism of initiation of protein synthesis.\textsuperscript{[4]} The initial codon–anticodon interaction occurs directly without the presence of the large subunit. Exactly how the correct tRNA binds to the mRNA without the presence of the ribosome is still unclear.\textsuperscript{[2]}

Fidelity in protein synthesis is contributed by several mechanisms, i.e., fidelity in mRNA transcription, tRNA aminoacylation, and codon–anticodon interaction. The cognate codon–anticodon interaction is followed by the accommodation of the aa-tRNA in the A-site of the ribosome due to the induced fit mechanism.\textsuperscript{[5–7]} Compared to the cognate anticodon, the rate of GTP hydrolysis and accommodation is significantly lower for the near/noncognate anticodons, which results in their rejection from the ribosome.\textsuperscript{[5]} This process, i.e., the kinetic proofreading, plays a major role in the fidelity of protein synthesis. However, it is not clear whether or not a misacylated tRNA influences this conformational change and whether or not it is affected by kinetic proofreading process during peptide bond formation.\textsuperscript{[7,8]}

The conformational change of the tRNA in the PTC is also independent of the codon–anticodon interaction since no fundamental differences are observed between the PTCs of the 70S and 50S structures during interaction with the full aa-tRNA and the small oligonucleotide, respectively.\textsuperscript{[4]} It is worth mentioning that this is not in conflict with the idea behind kinetic proofreading since the kinetic proofreading is based on the kinetic differences between the accommodation process of cognate and non/near-cognate codon–anticodon interaction rather than the structural differences. Moreover, the tRNA stabilization in the A-site of the ribosome after the EF-Tu dissociation is believed to be partly affected by the nature of the amino acid it is attached to. Though, whether this arises from the ribosomal affinity to the amino acid side chain itself or the correct acylated tRNA remains a dilemma, since the interaction of the amino acid side chain with ribosomal bases is not clearly observed.\textsuperscript{[4]} Thus, we assume that this stability is independent of the amino acid’s interaction with ribosomal bases or ribosomal proteins. This assumption is based on what has been reported regarding nonsequence-specific ribosomal interaction with aa-tRNA.

In addition, the fact that the ribosome might specify the amino acid side chain in the A-site is not applicable for initiator tRNA\textsubscript{i \text{met}} since it initially binds to the small subunit and the mRNA with the absence of large subunit. Furthermore, the initiator tRNA\textsubscript{i \text{met}} initially interacts with the P-site of the large subunit rather than the A-site. Thus, the fidelity of protein synthesis for a misacylated tRNA might be due to the different function of the tRNA structure itself. To investigate these assumptions, we analyzed the structure...
of six different tRNAs attached to both cognate and noncognate amino acids using computer simulation. Considering the structural differences between the initiator tRNA_{i}^{\text{met}} and elongator tRNA_{e}^{\text{met}}, we used both structures in our calculations. The energetic differences between these structures indicate that only the initiator tRNA_{i}^{\text{met}} behaves in a different manner when attached to a noncognate amino acid.

It is somehow impossible to use quantum mechanics methods to study the system due to the large size of the tRNA molecule. In order to deal with the large molecules with reasonable cost of calculation and accurate results, one can use hybrid methods, which will allow the combination of two or more computational techniques in one calculation.\cite{9-12} Most of these methods can only combine quantum mechanics (QM) and molecular mechanics (MM), called in general QM/MM methods. There are a few hybrid methods which can combine QM with QM as well as MM methods. One of the most famed methods from this region is known as ONIOM (Our own N-layered integrated molecular orbital and molecular mechanics) method, which may contain two or more different methods.\cite{9} This method divides the system into several onion-like layers and uses a proper computational chemistry method on each layer based on its size and role in the chemical reaction.\cite{13} One example is using quantum methods to study the bond breaking/forming process in the active site of an enzyme,\cite{14,15} while the rest of the molecule is studied using the MM method. In this study, the amino acid attached to the adenosine in the aminoacyl-arm of the tRNA is handled with a QM method while the rest of the tRNA is handled with the MM method.

**COMPUTATIONAL METHODS**

In this study, we used the X-ray crystal structures of six different tRNAs (i.e., tRNA_{i}^{\text{met}}, tRNA_{e}^{\text{met}}, tRNA_{\text{arg}}, tRNA_{\text{glu}}, tRNA_{\text{gln}}, and tRNA_{\text{phe}}),\cite{16-21} which are available in the Protein Data Bank (ID codes: 1YFG, 2CSX, 1F7V, 1G59, 1QRS, and 1EVV, respectively). These structures are used as the starting geometries of all calculations. All computations were carried out using the Gaussian 09 program. Molecular visualization was facilitated with the use of Gauss view.\cite{22}

Using the full fragment of the tRNA molecule in our calculations results in a large molecular size (near 2500 atoms). In order to have accurate yet computationally feasible calculations, we employed the ONIOM method,\cite{9} which is used for large systems since it enables one to divide the system into two (high and low) or three (high, medium, and low) different layers that can be treated with different levels of theory. A 2-layer ONIOM(QM/MM) calculation is used in this study where the amino acid arm of the tRNA is treated with the MP2 (second-order Møller–Plesset perturbation theory)
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Figure 1 ONIOM partitioning model: QM region is the active site of aa-tRNA molecule in which peptide bond formation occurs and MM region is the rest of the tRNA molecule.

We used the link atom model for covalent interaction between the two regions and the resulted dangling bond from the partitioning is capped with the hydrogen atom. The electrostatic interaction between the MM and QM regions is handled with electronic embedding (EE) where the partial charges from the MM region are included in the QM Hamiltonian. This brings more accuracy to the calculations compared to the mechanical embedding (ME), which is used in Gaussian by default.

The energy expression for the ONIOM(QM/MM) method is:

\[ E_{\text{ONIOM}} = E_{\text{Model,High}} + E_{\text{Real,Low}} - E_{\text{Model,Low}} \]  

where \( E_{\text{Model,High}} \) is the energy of the QM region with a high level of calculation, \( E_{\text{Real,Low}} \) is the energy of the MM region with a low level of calculation, and \( E_{\text{Model,Low}} \) is the energy of the QM region with the low level of calculation (similar to the one used in \( E_{\text{Real,Low}} \)). In a 2-layer ONIOM calculation for large molecules, the classical molecular mechanics method is normally used for the larger portion of the system and \textit{ab-initio} calculations for the site of interest. Defining a proper layer is important in ONIOM calculations. The general rule, which is common in all ONIOM calculations, is to use the high level of theory for the chemically active region (i.e., the model system),
while the low level of theory is used for the whole molecule (i.e., the real system).

Additional to this general rule, there are three important rules to define a proper layer (partitioning) in ONIOM calculations:

1. Bond breaking/formation must take place in the model system.
2. The boundaries between regions should not fall on double/triple chemical bonds. Likewise, all atoms in a ring must remain in the same region.
3. In a chemical reaction, all of the parameters in the MM region must be the same in both reactants and products. In this case, it is safer to extend the model system at least three bonds away from where the reaction is taking place.

In order to have accurate results, it is necessary to follow all these rules. Furthermore, the S-value (substituent-value) test can also validate either the aptness of the ONIOM (QM:MM) partitioning or the accuracy of the ONIOM approximation for a specific molecule and/or reaction. These two purposes are satisfied with two different methods. For the latter, Gaussian provides the keyword ONIOM = S-value. This purpose is sometimes necessary for verifying the accuracy of the ONIOM approximation for some molecules. However, the S-value test for the latter requires a high-level calculation on the real system which makes it computationally expensive and is not advised to be used for biomolecules (Equation 2)

$$\Delta S_{\text{ONIOM-approx}} = \{E_{\text{high}}(\text{Real}) - E_{\text{high}}(\text{Model})\} - \{E_{\text{low}}(\text{Real}) - E_{\text{low}}(\text{Model})\}. \quad (2)$$

The former, on the other hand, requires four MM calculations (Equation 3). It verifies whether or not the partitioning is appropriate for a specific structure and the chemical reaction.

$$\Delta S_{\text{MM-partition}} = \{E_{\text{MM}}(\text{react, Real}) - E_{\text{MM}}(\text{react, Model})\} - \{E_{\text{MM}}(\text{pro, Real}) - E_{\text{MM}}(\text{pro, Model})\}. \quad (3)$$

Since this study is not focusing on the chemical reaction itself, there is no necessity for carrying out the S-value test. Therefore, we followed the rule number 2 for extending the QM region in the aminoacyl-tRNA molecule.

Using different substitutions in a molecule, which can act as a “field” to distort the system’s charge distribution, enables us to study electronic effects on the system. We have applied this distortion to the aa-tRNA molecules by substituting the hydrogen atom of the $\alpha$-carbon ($C_{\alpha}$) of the amino acid moiety with halogen atoms. Each of the six different tRNAs were attached to five different amino acids (we used the same methionine structure for both
tRNA\textsubscript{Met} and tRNA\textsubscript{Met}), one cognate and four noncognate to that specific tRNA. This results in 30 different aminoacyl-tRNAs of which 6 are cognate and 24 are noncognate structures. Different substitutions on the amino acid moiety apply different electronic conditions to the molecule. The first substitution is the halogen atom substitution where the hydrogen of the C\textsubscript{\(\alpha\)} is substituted with four different halogen atoms, giving us a total of 150 substituted and nonsubstituted aa-tRNA structures (Figure 2A). The overall comparison is based on the increase of electronegativity from hydrogen to fluorine (H < I < Br < Cl < F).

The second type of substitution concerns the electron donating/withdrawing groups. Methyl (electron donating), ethyl (electron donating), and phenyl (electron withdrawing) substitutions were used for this purpose. In this substitution, the three mentioned groups substitute the hydrogen atom attached to the amino nitrogen (N8) (similar to the structure of N-alkylamino acids in\textsuperscript{[23]}). In order to apply the distortion in the charge distribution of the molecule, the three substituents are initially bonded to the N8, replacing only one of the hydrogen atoms and then another group (phenyl, ethyl, or methyl) is added to the system, replacing the second hydrogen atom attached to N8 (Figure 2B). The normal condition where both hydrogen atoms are attached to N8 in the amino group of the amino acid moiety is then compared with these six substitutions. These calculations are carried out for the six different tRNA molecules where each one is attached to five different amino acids resulting in an overall number of 210 substituted and nonsubstituted aa-tRNA structures.

We performed single point energy (SPE) calculations using ONIOM (MP2/6-31G (d,p):UFF) with no geometry optimization on the full fragment of the tRNA molecule. On the other hand, the geometry of the amino acid
molecule is optimized at the MP2 level before it is attached to the tRNA molecule. The geometry of this attachment is based on the geometry of the previously optimized aminoacyl-tRNA with molecular mechanics methods.

RESULTS AND DISCUSSION

The aim of this study is to investigate the structural differences between cognate and noncognate aminoacyl-tRNAs. Knowing the difference between the initiation and elongation processes in terms of fidelity made us involve the initiator tRNA\textsubscript{met} in the comparison. We used electronegativity to distort the charge distribution of the active site of the system where ester bond dissociation and peptide bond formation occur.

Effects of High Electronegativity on aa-tRNA Molecules

The influence of halogen substitutions on cognate and noncognate amino acids attached to tRNA reveals useful information on the behavior of these molecules under different conditions. The high electronegativity of these atoms affects the charge distribution of the atoms around the ester bond, which is of our interest. Therefore, in order to reduce computational time, we have measured the charge distribution of a short fragment of aminoacyl-tRNA (i.e., alanyl-ribose) with these atomic substitutions in order to have a better understanding of the effect of electronegativity on the surrounding atoms. Figure 3 shows the unsubstituted and four halogenic substitutions for the alanine amino acid attached to the ribose of Adenosine (excluding the purine base). The first structure is the unsubstituted alanine. We then apply these substitutions on the full fragment of cognate and noncognate aminoacyl-tRNAs to observe the behavior of these molecules under different substitutions.

As shown in Figure 3, there is a polar ester bond between the oxygen of the ribose sugar (O11) and the carbon of the amino acid moiety (C10). This is due to the high electronegativity of the O11 (i.e., 3.4) compared to the carboxylic carbon (C10). However, halogenic substitutions cause a more polar ester bond in this molecule with the exception of fluorine substitution (F25) (which results in the least polar C10–O11 ester bond).

Table 1 illustrates the charge values on C\textsubscript{\alpha}9, C10, and O11 for different halogenic substitutions (F, Cl, Br, and I) as well as the one without substitution (H).

As observed in this table, the least polar ester bond (the bond between C10 and O11) in the molecule is related to fluorine substitution (F25). On the other hand, the ester bond polarities related to Cl25 and Br25 are higher than that of F25. Iodine substitution results in the most polar ester bond in the system compared to the others. In this case, the relatively high negative charge on C\textsubscript{\alpha}9 results from the high nuclear charge of I25, which acts in
an electropositive way to Cα9. The higher electropositivity results in a lower ionization energy, which is also observed from the calculated first ionization energies of the alanine–ribose structure for all five conditions based on Koopmans’ theorem. As observed in Table 2, the lowest ionization energy is observed for the iodine substitution and the highest is observed for fluorine and hydrogen (unsubstituted).

**TABLE 1** Atomic charges in the molecule for different substitutions

<table>
<thead>
<tr>
<th>Substituents</th>
<th>Cα9</th>
<th>C10</th>
<th>O11</th>
</tr>
</thead>
<tbody>
<tr>
<td>H25 (0.210)</td>
<td>−0.06</td>
<td>0.821</td>
<td>−0.631</td>
</tr>
<tr>
<td>I25 (0.271)</td>
<td>−0.401</td>
<td>1.021</td>
<td>−0.700</td>
</tr>
<tr>
<td>Br25 (−0.087)</td>
<td>−0.026</td>
<td>0.868</td>
<td>−0.616</td>
</tr>
<tr>
<td>Cl25 (−0.004)</td>
<td>−0.065</td>
<td>0.906</td>
<td>−0.618</td>
</tr>
<tr>
<td>F25 (−0.395)</td>
<td>0.542</td>
<td>0.784</td>
<td>−0.623</td>
</tr>
</tbody>
</table>
To observe the behavior of cognate and noncognate aminoacyl-tRNAs under different charge distribution and ester bond polarity, we have performed the same halogenic substitutions on the amino acid attached to the full fragment of tRNA. The H25 is substituted with each of the four halogen atoms (i.e., F, Cl, Br, and I) after optimization of the amino acid attached to the adenosine in order to keep the structure the same for all substitutions.

Since the movement of aminoacyl-tRNAs in the ribosome is via the interactions between tRNA and ribosomal bases (mainly purine–pyrimidine hydrogen bonds), the overall energy of the molecule is not constant and varies based on the location (A-site or P-site) and on the state (hybrid or normal) of the molecule in the ribosome. Thus, the important issue under study is the relative energy variation, which is related to the behavior of the cognate and noncognate aminoacyl-tRNAs under different distortion fields within the system. The relative energy variation with respect to increase in electronegativity for different aminoacyl-tRNA molecules is shown in Figures 4A–4F. The second vertical axis on the right-hand side of Figure 4A is for the relative energy of the cognate met-tRNA\textsubscript{imet}, which is too large in scale to show in the same scale with the noncognate ones. This is most severe for iodine substituents on the cognate met-tRNA\textsubscript{imet}. As seen in Table 2, the lowest ionization energy on the molecule is caused by the iodine substituent which results in a relatively “unstable” structure. Moreover, the highly polar ester bond caused by iodine substitution makes the structure more likely to undergo chemical reactions. However, the highly polar ester bond only affects met-tRNA\textsubscript{i}\textsuperscript{met} (Figure 4A). Other aminoacyl-tRNAs show similar behavior for different substitutions. Furthermore, the met-tRNA\textsubscript{i}\textsuperscript{met} molecule is more stable (lower energy) compared to the misacylated tRNA\textsubscript{i}\textsuperscript{met} structures as well as to cognate and noncognate elongator aminoacyl-tRNAs under unsubstituted condition.

Since the ester bond is highly labile,[24] the stability of the met-tRNA\textsubscript{i}\textsuperscript{met} under normal unsubstituted condition prevents the cognate methionine from being hydrolyzed. However, this stability is not observed for the cognate elongator aminoacyl-tRNAs. This indicates that there is a difference between the structure of initiator and elongator tRNAs and the way they function under different conditions.

### Table 2

<table>
<thead>
<tr>
<th>Substituent</th>
<th>Electronegativity</th>
<th>Ionization energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>2.2</td>
<td>0.339</td>
</tr>
<tr>
<td>I</td>
<td>2.66</td>
<td>0.243</td>
</tr>
<tr>
<td>Br</td>
<td>2.96</td>
<td>0.306</td>
</tr>
<tr>
<td>Cl</td>
<td>3.16</td>
<td>0.329</td>
</tr>
<tr>
<td>F</td>
<td>3.98</td>
<td>0.339</td>
</tr>
</tbody>
</table>
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**FIGURE 4** Relative energy variation for cognate and noncognate aa-tRNAs with halogen substitutions on their amino acid moiety: (A) The energy variation for the initiator tRNA$^\text{met}$ attached to the five different amino acids. (B–F) The energy variation for the other five elongator tRNA structures attached to the same amino acids.

**Effect of Electron Donating/Withdrawing Groups on aa-tRNA**

Based on the effect of methyl groups on the rate of peptide bond formation$^{[23]}$ and to further investigate the behavior of the cognate and noncognate aminoacyl-tRNAs under different substitutions, alkyl and phenyl groups were introduced in the amino side of the amino acid moiety.

In this section, we study the effects of substitution on the charge distribution of the cognate and noncognate aminoacyl-tRNAs by substituting electron donating groups (EDG) and electron withdrawing groups (EWG) on the hydrogen atoms attached to the amino nitrogen of the amino acid moiety. Methyl, ethyl, and phenyl groups were used as substituents on both
hydrogen atoms of the amino nitrogen. Based on the atom these groups are attached to (i.e., N8), each of these groups acts as either EDG or EWG. That is, the methyl and ethyl groups have an electron donating inductive effect (+I) on the amino acid moiety whereas the phenyl group has an electron withdrawing mesomeric effect (−M). All seven systems are calculated for the six different tRNAs attached to their cognate amino acids and four different noncognates (Figures 5 and 6). The strength of electron donation is determined by the increase in the N8 negative charge of the amino acid moiety. The electron-withdrawing substitutions result in smaller negative charges on N8. The N8 negative charge is near zero in the presence of di-phenyl substituent.

In Figure 5, we compare the relative energy variation of cognate and noncognate aminoacyl-tRNAs. Overall, similar behavior is observed when each tRNA is attached to cognate and noncognate amino acids (especially in tRNA Gin). As mentioned in Section 3.1, our goal in this study is to observe the relative energy variations of cognate and noncognate aa-tRNAs under different conditions and as observed in Figure 5, they are almost similar for elongator tRNAs. On the other hand, we found the initiator tRNA Met behavior to be different when attached to noncognate amino acids (Figure 6).

In contrast to elongator tRNAs, met-tRNA Met is more stable under normal condition with no substitution, which is imperative to protect the methionine from being hydrolyzed when met-tRNA Met is attached to the complex of mRNA-smaller ribosomal subunit before attachment to the larger ribosomal subunit. During the elongation process, this role is played by the universally
conserved rRNA base U2585, which protects the peptidyl tRNA’s ester bond from being hydrolyzed when the A-site is empty\(^4\) (after translocation), and the initiator met-tRNA\(_i\)^{met} lacks this protection when it is not yet bound to the larger ribosomal subunit. Such behavior is similar to that for the electronegative atomic substitutions discussed in Section 3.1. The unsubstituted amino acid structure is most stable for cognate met-tRNA\(_i\)^{met} compared to the other structures (Figure 4).

The arginine amino acid, on the other hand, with a basic side chain and positive charge, is expected to attract electrons from the active site (i.e., the carboxyl group attached to the ribose) and results in a more stable molecule. Moreover, the presence of EDG in the amino side of arginine results in a positive charge at the other end of the arginine moiety (i.e., the carboxyl group). However, this amino acid behaves in a different way when attached to the tRNA\(_i\)^{met}. Arg-tRNA\(_i\)^{met} is relatively unstable in the presence of EDG and under a normal unsubstituted condition. This would ultimately lead to the hydrolysis of the structure and dissociation of the noncognate amino acid from the initiator tRNA\(_i\)^{met}. The situation is almost the same for gln-tRNA\(_i\)^{met} (excluding the steric effect of di-phenyl substitution) and phe-tRNA\(_i\)^{met}. It is in contrast with the elongator tRNA\(_i\)^{met}, where its attachment to the noncognate amino acids results in highly stable structure in the presence of EDG as well as EWG. These different behaviors of tRNA\(_i\)^{met} for different amino acids compared to the elongator tRNAs indicate the importance of the amino acid side chain identity for the initiator tRNA\(_i\)^{met}. 

![Figure 6](https://example.com/figure6.png) 

**FIGURE 6** Relative energy variation for the cognate and noncognate aa-tRNA\(_i\)^{met} with electron donating/withdrawing substitutions on the amino nitrogen.
CONCLUSION

Based on previous biochemical studies on the structure and function of the ribosome, it is believed that the type of amino acid side chain affects the stability of the tRNA binding at the A-site of the ribosome after the EF-Tu dissociation. However, it is not clear whether the amino acid side chain interacts with the ribosomal bases at the A-site. In this study, we observed the specificity of initiator tRNA$_{\text{met}}$ toward its cognate amino acid side chain (i.e., methionine). This specificity does not exist in elongator tRNAs. Knowing that the advantage of elongator tRNAs over tRNA$_{\text{met}}$ is their interaction with the ribosomal A-site, the results in this study demonstrate the importance of the larger ribosome in stabilizing the correct acylated tRNA at the A-site. Furthermore, the initiator tRNA$_{\text{met}}$ binds initially to the P-site of the larger ribosome, the site that is less likely to bind to the amino acid side chain compared to the A-site. Therefore, the difference between initiator tRNA$_{\text{met}}$ and other elongator tRNAs is in the latter’s tendency to be stabilized at the ribosomal A-site, and in the former’s less likeliness to be stabilized because of its initial attachment to the P-site. This indicates the lack of substrate specification at the P-site, which agrees well with existing biochemical data.

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