Mechanism of the activation step of the aminoacylation reaction: a significant difference between class I and class II synthetases

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In the present work we report, for the first time, a novel difference in the molecular mechanism of the activation step of aminoacylation reaction between the class I and class II aminoacyl tRNA synthetases (aaRSs). The observed difference is in the mode of nucleophilic attack by the oxygen atom of the carboxylic group of the substrate amino acid (AA) to the αP atom of adenosine triphosphate (ATP). The syn oxygen atom of the carboxylic group attacks the α-phosphorous atom (αP) of ATP in all class I aaRSs (except TrpRS) investigated, while the anti oxygen atom attacks in the case of class II aaRSs. The class I aaRSs investigated are GluRS, GlnRS, TyrRS, TrpRS, LeuRS, ValRS, IleRS, CysRS, and MetRS and class II aaRSs investigated are HisRS, LysRS, ProRS, AspRS, AsnRS, AlaRS, GlyRS, PheRS, and ThrRS. The variation of the electron density at bond critical points as a function of the conformation of the attacking oxygen atom measured by the dihedral angle ψ (Cα–C′) conclusively proves this. The result shows that the strength of the interaction of syn oxygen and αP is stronger than the interaction with the anti oxygen for class I aaRSs. This indicates that the syn oxygen is the most probable candidate for the nucleophilic attack in class I aaRSs. The result is further supported by the computation of the variation of the nonbonded interaction energies between αP atom and anti oxygen as well as syn oxygen in class I and II aaRSs, respectively. The difference in mechanism is explained based on the analysis of the electrostatic potential of the AA and ATP which shows that the relative arrangement of the ATP with respect to the AA is opposite in class I and class II aaRSs, which is correlated with the organization of the active site in respective aaRSs. A comparative study of the reaction mechanisms of the activation step in a class I aaRS (Glutaminyl tRNA synthetase) and in a class II aaRS (Histidyl tRNA synthetase) is carried out by the transition state analysis. The atoms in molecule analysis of the interaction between active site residues or ions and substrates are carried out in the reactant state and the transition state. The result shows that the observed novel difference in the mechanism is correlated with the organizations of the active sites of the respective aaRSs. The result has implication in understanding the experimentally observed different modes of tRNA binding in the two classes of aaRSs.

Keywords: aminoacylation; aminoacyl tRNA synthetase; class I aaRS; class II aaRS

Introduction

Protein biosynthesis takes place in successive stages such as aminoacylation, initiation, elongation, termination, release, folding, and posttranslational processing (Berg, Tymoczko, & Stryer, 2002; Nelson & Cox, 2002). The relationship between an amino acid (AA) and its cognate tRNA is established in the aminoacylation reaction. This vital reaction links the realm of the protein and the RNA world. The reaction occurs in two steps (Cavarelli, Delagoutte, Eriani, Gangloff, & Moras, 1998; Delarue, 1995; Perona, Rould, & Steitz, 1993). The first step is the AA activation with the formation of aminoacyl adenylate and release of inorganic pyrophosphate (Figure 1). The second step is the charging of tRNA in which the aminoacyl group is transferred to the tRNA. Both activation and charging of 20 natural AAs are catalyzed by 20 aminoacyl tRNA synthetases (aaRSs).

The aaRSs are categorized into two classes (class I and class II) which have several differences (Arnez & Moras, 1997; Cavarelli et al., 1998; Delarue, 1995; Ibba & Söll, 2000; Perona et al., 1993). The surprising speed and accuracy of the aminoacylation reaction is a long-standing puzzle. Unless the reaction mechanism within the active site is understood in molecular detail, it is not possible to resolve these problems. It is well known that the reaction mechanism is best understood using quantum mechanical methods. Despite the wealth of informa-
Step 1: Amino acid activation: Amino acid (AA) + Adenosine triphosphate (ATP) + aminoacyl-tRNA synthetase (aaRS) = aminoacyl-adenylate (AA-AMP) + inorganic pyrophosphate (PPi)

\[
\begin{align*}
\text{H}_3\text{N}-\text{C} & - \text{R} \\
\text{O} & - \text{O} \\
\text{O} & - \text{P} \\
\text{N} & - \text{N} \\
\text{N} & - \text{N} \\
\text{NH}_2 & \\
\text{OH} & - \text{OH} \\
\text{OH} & - \text{OH} \\
\text{O} & - \text{P} \\
\text{O} & - \text{P} \\
\text{O} & - \text{P} \\
\text{H}_3\text{N} & - \text{C} \\
\text{R} & - \text{NH}_3 \\
\text{C} & - \text{O} \\
\text{O} & - \text{P} \\
\text{N} & - \text{N} \\
\text{N} & - \text{N} \\
\text{NH}_2 & \\
\text{OH} & - \text{OH} \\
\text{OH} & - \text{OH} \\
\text{O} & - \text{P} \\
\text{O} & - \text{P} \\
\text{O} & - \text{P} \\
\end{align*}
\]

Step 2: tRNA charging: AA-AMP + aaRS + tRNA = aminoacyl-tRNA (AA-tRNA) + Adenosine monophosphate (AMP) + aaRS

Figure 1. The reaction scheme of the first step (activation step) and second step (tRNA charging) of aminoacylation reaction.

The studies aimed at understanding the reaction mechanism from electronic structure-based analysis are limited (Dutta Banik & Nandi, 2009, 2010, 2011; Liu & Gauld, 2008; Nandi, 2011; Ramalho, Caetano, da Cunha, Souza, & Rocha, 2009). A significant difference in the reaction mechanism of the second step of aminoacylation reaction between the class I and class II aaRSs is already known. The class I enzymes attach the corresponding AA to the 2′-OH group of 3′-terminal adenosine of tRNA and the class II enzymes (except PheRS) attach the AA to the 3′-OH group of adenosine of tRNA (Ibba & Söll, 2000). To the best of our knowledge, no difference at the molecular level in the reaction mechanism of the activation step of aminoacylation reaction between the class I and class II aaRSs is reported in the literature. The aim of the present study is to analyze the molecular details of the mode of nucleophilic attack in the first step of aminoacylation reaction for the class I and class II aaRSs using electronic structure based method.

It is proposed, based on crystallographic studies that the activation step follows an inline displacement mechanism in which the oxygen atom of the carboxylic acid group of AA attacks to the α-phosphorous atom (αP) of the ATP for both class I and class II aaRSs. The common reaction mechanism of the activation step of aminoacylation reaction is suggested for both class I and class II aaRSs.
II aaRSs (Cavarelli et al., 1998; Delarue, 1995; Perona et al., 1993) as shown schematically in Figure 1. Electronic structure based analysis also confirmed the mechanism of the activations step (Dutta Banik & Nandi, 2009, 2010, 2011; Nandi, 2011) and tRNA charging step (Liu & Gauld, 2008) for HisRS. In principle, two oxygen atoms of the carboxylic acid group of the substrate AA may adopt four probable conformations with respect to α-amino group of AA. Such probable conformations are syn-periplaner, sp; syn-clinal, sc (collectively denoted as syn) and anti-clinal, ac; and anti-periplaner, ap (collectively denoted as anti) (Eliel & Wilen, 1993). The carboxylic oxygen making nucleophilic attack may have any conformations in the above mentioned ranges such as sp, sc, ac, or ap during the reaction. The preferred conformation of the attacking oxygen can be followed from the preferred interaction between the attacking nucleophile and the electrophilic center. The interaction between the two atoms can be understood from the analysis of topology of electron density, \( \rho \) using robust quantum mechanical method (‘Atoms in Molecule’ theory or AIM theory) (Bader, 1991; Sjoberg & Politzer, 1990; Wiberg, Bader, & Lau, 1987). The presence of bond critical point (BCP) and bond path connection between two nuclei indicates an interaction which can be computed using AIM theory. The electron density at BCP \( \rho_b \) is used as a measure of the strength and the nature of the interaction (covalent, nonbonded or electrostatic) can be followed from the Laplacian of the electron density at BCP (Bader, 1991).

In the present work, we calculated the variation of \( \rho_b \) between the oxygen atom of the carboxylic acid group and the αP of ATP as a function of the conformation of the attacking oxygen atom. This is measured by the dihedral angle \( \psi \) (C\(^\alpha\)-C') or \( \psi \) (N–C\(^\alpha\)-C'–O) following standard convention (Adrian-Scotto & Vasilescu, 2008; Altona & Sundaralingam, 1972; IUPAC-IUB Nomenclature, 1970; Saenger, 1984). The \( \psi \) (C\(^\alpha\)-C') represent the dihedral angle between the plane containing nitrogen atom of α-amino group, chiral carbon atom, carbonyl carbon atom of carboxylic group; and the plane containing chiral carbon atom, carbonyl carbon atom of carboxylic group, oxygen atom of carboxylic group of substrate AA (Figure S1). The variation includes conformations such as sp, sc, ac, or ap. The conformation of the carboxylic oxygen atom having strongest interaction with the αP of ATP should have the higher value of \( \rho_b \) compared to the other conformations. The computation is carried out for both class I and class II aaRSs. The result is supported by the calculation of the variation of interaction energy between syn oxygen atom of AA and αP atom of ATP and that between the anti oxygen of AA and αP atom of ATP as a function of mutual separation in the reactant state for class I and class II aaRSs. To understand the mode of nucleophilic attack as well as the influence of the active site organization on the mode of nucleophilic attack in class I and class II aaRSs, a comparative study of the reaction mechanisms of the activation step in a class I aaRS (GlnRS) and in a class II aaRS (HisRS) is carried out by transition state analysis. The AIM analysis of the interaction between active site residues or ions and substrates is carried out in the reactant state and the transition state.

Since the interaction between the AA and ATP is principally electrostatic in nature, the origin of the difference in the mode of attack (if any) can be followed from the analysis of the electrostatic potential near the reaction center. The difference in the mode of nucleophilic attack between various aaRSs is explained using electrostatic potential analysis (ESP) of the substrates (Popelier, 1998). The aminoacylation reaction being a nucleophilic reaction, the population difference \( \delta q = q_p - q_o \) between the αP atom of ATP and oxygen atom of carboxylic acid group (the attacking atom of the nucleophile closest to the αP) can serve as a measure of the propensity of the nucleophilic reaction. The influence of the charge distributions of neighboring active site residues and ions favor the nucleophilic attack; and the computation of \( \delta q \) in presence and in absence of active site residues is expected to reveal the influence of the active site residues on the progress of the reaction.

The results are interesting and reveal a significant difference in the mode of nucleophilic attack between the class I and class II aaRSs for the first time. The syn oxygen atom of the carboxylic group attacks the αP of ATP in class I aaRSs, while the anti oxygen atom attacks in the case of class II aaRSs. The present study shows that the mutual arrangements of the AA and ATP in class I and class II are such that modes of the approaches of the oxygen atoms for nucleophilic attack in the two cases need to be opposite. The analysis of the transition state using quantum mechanical/semi-empirical (QM/SE) method and \( \delta q \), respectively show that the subtle difference in mode of attack is correlated with the striking difference in the active site organization among the two classes of aaRSs which remained enigmatic till date.

The better efficiency of the enzymatic reaction over the same reaction occurring in bulk solvent is a subject of much interest (Warshel et al., 2006). In the bulk solvent, the syn or the anti oxygen attacks are equally probable on an average. Further, the adenylate geometries resulting from syn or anti attack are free for conformational rearrangements in bulk solvent. In contrast, interactions between syn oxygen and active site residues are not equivalent with those between the anti oxygen and active site residues. This is due to the spatial heterogeneity and dissymmetry of the organization of active site. Similarly the product structure of the adenylate in each aaRS is stabilized and retained by the active site and conformational rearrangement within the enzyme is less
probable than in the bulk. The aforesaid interaction pattern observed between active site residues of aaRS and substrates (Nandi, 2011) are described in section A of the supplementary material. The difference in the reaction mechanism between class I and class II aaRSs is expected to be correlated with the organization of the active site structure of the respective aaRS. However, studies are unavailable at present. This unresolved problem provides further impetus in the present study. In the following section, we present the computational methods.

**Methods**

The available crystal structures of class I and class II aaRSs of the reactant state as well as adenylate state of activation step of aminoacylation reaction are used in the present work. Class I aaRSs considered in the reactant states are GluRS, GlnRS, TyrRS, and TrpRS; and class II aaRSs considered are HisRS, LysRS, and ProRS. Class I aaRS considered in the adenylate state are CysRS, GluRS, GlnRS, LeuRS, IleRS, MetRS, TyrRS, and ValRS; and class II aaRSs considered are AlaRS, AspRS, AsnRS, GlyRS, HisRS, PheRS, and ThrRS. Details of the model built are described in the introduction section (shown in Figure S1). The oxygen atom closest to the αP atom in reactant state is the probable candidate to attack the αP atom of ATP. These pair of oxygen atoms are referred to as O(1) and O(2) for convenience and the numbering is arbitrary. The oxygen atoms have conformations such as sp, sc, ac, or ap which are denoted by the ψ (Cα–C′) dihedral angle as mentioned in the introduction section (shown in Figure S1). The oxygen atom closest to the αP atom in reactant state is the probable candidate to attack the ATP. The separation between the pair of oxygen atoms of the carboxylic acid group of substrate AA and αP atom of ATP in reactant state is measured and the conformation of corresponding oxygen atoms is noted (Table 1 of the supplementary material).

The attacking oxygen atom of the carboxylic acid group of substrate AA develops bonding with the αP atom of ATP in the product state and not with the other oxygen. Hence, the geometry of the adenylate state is suggestive of the conformation of the attacking oxygen (shown in the Table 2 as well as Figure S2 and S3, respectively).

As mentioned in the introduction, $\rho_b$ can be used as a measure of the strength of interaction and its nature (covalent, ionic, or electrostatic) can be followed from the Laplacian of the electron density at the critical point (Bader, 1991) from AIM theory. To confirm the strength of the interaction between each of the pair of oxygen atoms and the αP atom, we calculated the variation of $\rho_b$ between oxygen atom of the carboxylic acid group and αP of ATP (which indicates the strength of interaction) as a function of the ψ (Cα–C′) dihedral angle using AIM method. It is expected that among the pair of carboxylic oxygen in the adenylate state, the $\rho_b$ value would be greater for the bonded oxygen atom compared to the nonbonded one. The variation of $\rho_b$ with the conformation of oxygen atom is computed for various class I and class II aaRSs as shown in Figure 2 and Figure 3, respectively. The calculations are performed using HF/6–31G** level of theory.

To further explore the difference in the modes of nucleophilic attack in class I and class II aaRSs, we analyzed the variation of the interaction energy as a function of mutual separation ($\Delta R$) between the αP atom of ATP and the anti oxygen, and that between αP atom and the syn oxygen. The difference between the two interaction energies (denoted as $\Delta E_{anti-syn}$) as a function of $\Delta R$ is plotted in Figure 4 for all aaRSs. The variation represents the relative ease of approach of each oxygen towards the αP atom during the course of the reaction. The $\Delta E_{anti-syn}$ values in the reactant state are computed for various class I and class II aaRSs at the HF/6–31G** level of theory. We also included the effect of electron correlation in the computation using MP2/6–31G** level of theory (Figure 4(b)). The detailed results are shown in the figures S6 and S7 of the supplementary material for class I and class II aaRS, respectively.

We carried out the electronic structure-based analysis of the transition state of the activation step for a class I (GlnRS) and class II aaRS (HisRS). The QM/SE model for the GlnRS includes the ATP bound with one Mg2+ ion, substrate AA (Gln), the Pro32-Pro33-Gly34 tripeptide, the side chains of His40, His43, Asp66, Arg260, and Lys270 as well as the ribose ring of A76 of tRNA (total number of atoms present is 136). The model of HisRS includes the side chains of Glu83, Arg113, Gln127, and Arg259, two Mg2+ ions, two water molecules and the substrate AA (His), and ATP (total number of atoms present as 100). The model structures are optimized using two-level ONIOM (HF/6–31G*:PM3) method. The carboxylic acid group of Gln, αP atom of ATP with attached oxygen atoms, the imidazole group of His43, amino group of Lys270, and 2’OH group as well as 3’OH group of ribose ring of A76 of tRNA are included in ab initio level of theory in the model of GlnRS. Remaining atoms of the reactant and surrounding residues are considered at the semi-empirical level (PM3). In the case of HisRS, the carboxylic acid group of His, αP of ATP with attached oxygens, and the guanidinium group of Arg113 and Arg259 are included in the ab initio level of theory. Remaining atoms of the reactant and surrounding residues are considered at the semi-empirical (PM3) level. QST3 method is used for transi-
tion state calculation. The transition state is identified by
the presence of a single imaginary frequency. The sche-
matical representation of the transition state geometries of
GlnRS and HisRS are shown in Figure 5(a) and (b),
respectively. The Cartesian coordinates for GlnRS and
HisRS are given in Table 3(A) and (B), respectively.
AIM calculation for the reactant state and transition
state.

To understand the origin of the difference in the
mode of nucleophilic attack, the electrostatic potential
(ESP) at the molecular surface of ATP and AA (in
the absence of all active site residues) is computed. The
electrostatic potential, \( V(\vec{r}) \) that the electrons and nuclei
of a molecule create at each point \( \vec{r} \) in the surrounding
space is given by Equation (1).

\[
V(\vec{r}) = \sum_{\Lambda} \frac{Z_{\Lambda}}{|R_{\Lambda} - \vec{r}|} - \int \frac{\rho(\vec{r}')d\vec{r}'}{|\vec{r}' - \vec{r}|}
\]

(1)

\( Z_{\Lambda} \) is the charge of the nucleus \( \Lambda \), located at \( \vec{R}_{\Lambda} \).

The electronic density function of the molecule is
denoted by \( \rho(\vec{r}) \) (Popelier, 1998). The ESP analysis is
carried out for both class I and class II aaRSs based on
the crystal structure of the reactant state using HF/6–
31G** level of theory. The ESP surfaces for class I and
class II aaRSs are shown in Figure 6(a) and (b), respec-
tively.

The population difference (\( \delta q = q_p - q_o \)) between the
\( \alpha P \) atom of ATP (\( q_p \)) and oxygen atom of carboxylic
acid group (closest to the \( \alpha P \) and is the attacking atom
of the nucleophile) of substrate AA (\( q_o \)) in presence and
in absence of active site residues is computed using Mul-
liken population analysis scheme (MPA) and Natural population analysis scheme (Reed, Curtiss, & Weinhold, 1988; Reed, Weinstock, & Weinhold, 1985; Sun, Li, Zhang, Ma, & Liu, 2008). The NPA scheme utilizes the natural atomic orbital (NAO) and natural bond orbital. The natural population $q_i^A$ of orbital $\phi_i^A$ on atom A is the diagonal density matrix element in the NAO basis. Thus, $q_i^A = \langle \phi_i^A | \Gamma | \phi_i^A \rangle$, where $\Gamma$ is the density matrix and the atomic population $q(A)$ is given by the summation over all orbitals centered on the atom A and is given by, $q(A) = \sum_i q_i^A$. The population difference ($\delta q$) is measured for both class I and class II aaRSs in absence and presence of active site residues and Mg$^{2+}$ ion(s). The active site residues and ions influence the $\delta q$ and concomitantly dictate the progress of the nucleophilic reaction. The available crystal structures of the reactant state are used for the computation. The set of active site residues belong to the vicinity of the reaction center are included to understand the influence of active site on the propensity of nucleophilic reaction. The reaction center is the location of the nucleophilic attack between the carboxylic acid group of AA and the $\alpha$-phosphorous atom of ATP, denoted as $\alpha$. Further, the active site residues and ions near the $\beta$P and $\gamma$P region of ATP are also included in computation.

The residues considered in each aaRSs are as follows. The active site residues considered for GlnRS are Phe31, Pro32, Pro33, Glu34, His34, Asp66, Ile81, Arg260, Lys270, A76, and single Mg$^{2+}$ ion. For GluRS, Ile6, Ala7, Pro8, Ser9, Pro10, Asp13, Pro14, His15, Thr18, Glu41, Glu208, Trp209, Lys243, Lys246, Arg247, single Mg$^{2+}$ ion, and single H$_2$O are considered. For TrpRS, Ile8, Gln9, Glu107, Lys111, Lys192, Val143, Asp146, Glu147, His150, Lys195, Tyr125, single Mg$^{2+}$ ion, and two H$_2$O molecules are considered. The active site residues included for TyrRS are Ala44, Asp45, Thr47, Leu51, His52, His55, Gly81, Gly84, Asp85,
Table 1. The difference in charge population ($\delta q = q_p - q_o$), in a.u., of the $\alpha$-phosphorus atom of ATP $q_p$ and the carboxylic oxygen atom of substrate amino acid, $q_o$, for GlnRS, GluRS, TrpRS, TyrRS, HisRS, LysRS, and ProRS. The data represented in column A are in absence of all active site residues and in column B is in presence active site residues close to the region of nucleophilic attack using NPA and MPA schemes at HF/6–31G** level of theory. The corresponding models in column B include substrate AA, ATP and different active site residues mentioned in the third column.

| aaRS   | Class | The active site residues present in the corresponding models                                                                 | Population analysis scheme | $\delta q = q_p - q_o$ (a.u.) |
|--------|-------|------------------------------------------------------------------------------------------------------------------------------|-----------------------------|-----------------------------|
| GlnRS  | I     | Phe31, Pro32, Pro33, Glu34, His40, His43, Asp66, Ile81, Arg260, Lys270, A76, and single Mg$^{2+}$ ion                          | NPA                         | 3.63                        | 3.75                        |
|        |       |                                                                                                                                | MPA                         | 2.29                        | 2.55                        |
| GluRS  | I     | Ile6, Ala7, Pro8, Ser9, Pro10, Asp13, Pro14, His15, Thr18, Glu41, Glu208, Trp209, Lys243, Lys246, Arg247, single Mg$^{2+}$ ion, and single H$_2$O           | NPA                         | 3.64                        | 3.70                        |
|        |       |                                                                                                                                | MPA                         | 2.26                        | 2.57                        |
| TrpRS  | I     | Ile8, Gln9, Gln107, Lys111, Lys192, Val143, Asp146, Gln147, His150, Lys195, Tyr125, single Mg$^{2+}$ ion, and two H$_2$O molecules | NPA                         | 3.63                        | 3.71                        |
|        |       |                                                                                                                                | MPA                         | 2.14                        | 2.42                        |
| TyrRS  | I     | Ala44, Asp45, Thr47, Leu51, His52, His55, Gly81, Gly84, Asp85, Ser87, Arg93, Tyr108, Tyr175, Gly193, Gly194, Asp196, Gln197, Lys232, Lys235, and single Mg$^{2+}$ ion | NPA                         | 3.61                        | 3.75                        |
|        |       |                                                                                                                                | MPA                         | 2.21                        | 2.50                        |
| HisRS  | II    | Thr60, Val62, Pro82, Gln83, Gln84, Arg113, Arg121, Gln127, Arg259, Gly260, Leu261, Ala284, Ala306, Arg311, two Mg$^{2+}$ ion (Mg$^{2+}$_1, Mg$^{2+}$_2), and two H$_2$O molecules | NPA                         | 3.52                        | 3.73                        |
|        |       |                                                                                                                                | MPA                         | 2.16                        | 2.45                        |
| LysRS  | II    | Gly216, Ala217, Ser218, Ala238, Asn260, Arg262, Arg269, His270, Met276, Glu278, Val396, Ser397, Pro398, Arg412, Gln414, Asn424, Met455, Gly475, Arg480, three Mg$^{2+}$ ion (Mg$^{2+}$_1, Mg$^{2+}$_2, and Mg$^{2+}$_3), and 12 H$_2$O molecules | NPA                         | 3.56                        | 3.70                        |
|        |       |                                                                                                                                | MPA                         | 2.22                        | 2.56                        |
| ProRS  | II    | Thr11, Gln113, Arg142, Arg152, Trp158, Glu160, Phe205, Thr211, Thr212, The228, His230, Gly260, Arg264, and two Mg$^{2+}$ ion (Mg$^{2+}$_1, Mg$^{2+}$_2) | NPA                         | 3.52                        | 3.71                        |
|        |       |                                                                                                                                | MPA                         | 2.16                        | 2.46                        |
Ser87, Arg93, Tyr108, Tyr175, Gly193, Gly194, Asp196, Gln197, Lys232, Lys235, and single Mg$^{2+}$ ion. Similarly for HisRS, Thr60, Val62, Pro82, Glu83, Gly84, Arg113, Arg121, Gln127, Arg259, Gly260, Leu261, Ala284, Ala306, Arg311, Mg$^{2+}$1, Mg$^{2+}$2, and two H$_2$O molecules are considered. The active site residues such as Gly216, Ala217, Ser218, Ala238, Asn260, Arg262, Arg269, His270, Met276, Glu278, Val396, Ser397, Pro398, Arg412, Glu414, Asn424, Met455, Gly475, Arg480, Mg$^{2+}$1, Mg$^{2+}$2, Mg$^{2+}$3, and 12 H$_2$O molecules are considered for LysRS. In case of ProRS, Thr111, Glu113, Arg142, Arg152, Trp158, Glu160, Phe205, Thr211, Thr212, Thr228, His230, Gly260, Arg264, Mg$^{2+}$1, and Mg$^{2+}$2 are considered. The respective residues are also mentioned in Table 1. The population difference is computed using HF/6–31G** level of theory.

Both natural and MPA have been carried out. The results of population analysis are presented in Figure 7. Gauss-

Figure 6. Electrostatic potential on the molecular surfaces of ATP and substrate AA in (a) (i) GluRS, (ii) GlnRS (iii) TyrRS, and (iv) TrpRS and in (b) (i) HisRS, (ii) LysRS, and (iii) ProRS in the reactant state as present in the crystal structure of the corresponding aaRSs. The ESP calculations are performed using HF/6–31G** level of theory with isodensity value of 0.0004 a.u. The color variation ranges from red (electronegative) to blue (electropositive) with the ESP values ranging from $-0.6$ a.u ($-16.35$ eV) to $+0.6$ a.u. ($16.35$ eV).
The conclusions are valid for both oxygen atoms of the carboxylic acid group. The oxygen with syn conformation has the lowest probability to form the bond with the αP atom of ATP. The result indicates a novel difference in the mode of nucleophilic attack by the oxygen atom of the carboxylic group of substrate AA to the αP atom of ATP between class I and class II aaRSs.

Although TrpRS belongs to class I, the mode of nucleophilic attack in TrpRS is exceptional. In TrpRS, the anti oxygen atom acts as attacking oxygen during the reaction, whereas the syn oxygen of substrate AA is the most probable attacking conformation for all other class I aaRSs investigated. Trp has the largest side chain (Zamyatin, 1972). In case, the syn oxygen atom of Trp acts as attacking atom, such a mode of nucleophilic attack leads to unfavorable nonbonded interaction (short-range repulsion) between the side chain of Trp and sugar ring of ATP and does not occur for the same reason. In contrast, the anti conformation is suitable for the nucleophilic attack in this case. Consequently, the anti oxygen atom acts as the attacking atom during the activation step in case of TrpRS.

The foregoing results, which show two different and distinct modes of attack in the two classes of aaRSs, are further confirmed by studying the variation of interaction energy as a function of mutual separation (ΔR) between oxygen atoms of AA and αP atom of ATP. The variation of the interaction energy between the anti oxygen and αP atom, and that between the syn oxygen and αP atom are computed as a function of mutual separation (ΔR). The difference between the two energy quantities (denoted as ΔE_{anti-syn}) is the difference in interaction energies of the carboxylic oxygen atoms (with syn and anti) as they approach to the αP atom of ATP for nucleophilic attack. In other words, ΔE_{anti-syn} represents the relative ease of the anti or syn attack. Plots of ΔE_{anti-syn} as a function of ΔR for both classes of aaRSs are shown in Figure 4(a) and (b). The positive value of ΔE_{anti-syn} for class I aaRSs (except TrpRS) confirms that the approach of the syn oxygen atom is more favorable for class I aaRSs. The exceptional behavior in the case of TrpRS is...
due to its largest side chain, which prevents the approach of the syn oxygen as mentioned earlier. The negative value of $\Delta E_{\text{anti-syn}}$ for class II aaRSs confirms that the approach of anti oxygen atom is more favorable for class II aaRSs. The incorporation of electron correlation at the MP2 level of theory does not change the conclusions made based on HF/6–31G** level of theory (shown in Figure 4(b)). The effect of electron correlation is 0.21% of the total energy on an average (over all separations considered for various aaRSs) computed at the MP2/6–31G** level. The individual plots are shown in Figures S6 and S7 of the supplementary material. Thus, the variation in energy shown in Figure 4(a) and (b) strongly supports the conclusions made from the variation in electron density as shown in Figures 2(a), (b) and Figures 3 (a), (b), respectively.

The foregoing conclusion about the two different modes of attack is further corroborated by the observed conformations of the oxygen atoms in various class I and class II aaRSs based on the crystal structures. The separations between each of the oxygen atoms of carboxylic acid group of substrate AA and $aP$ atom of ATP in the reactant states are shown in the Table 1 in the supplementary material based on the corresponding crystal structures. Out of the four available crystal structures of class I aaRSs, the conformation of the closest oxygen is syn for three and anti for one (TrpRS). In contrast, the conformation of the closest oxygen is anti for all out of three available crystal structures of class II aaRSs. As mentioned before, the oxygen atom closer to the $aP$ atom of ATP is the most probable candidate for the attack. The result is confirmed from the conformation of the oxygen atom in the adenylate state of various aaRSs as shown in Table 2 in the supplementary material. Out of the eight available crystal structures of adenylate state of class I aaRSs, the conformation of the oxygen atom covalently linked with $aP$ (attacking oxygen) is sp for six and sc for two aaRSs. This is shown in Figure S2 in the supplementary material. In contrast, the conformation of the oxygen covalently linked with $aP$ (attacking oxygen) is ap for six and ac for three out of the nine crystal structures of adenylate state of class II aaRSs. This is shown in Figure S3 in the supplementary material. Consequently, the attacking oxygen atom of substrate AA in both reactant and adenylate state has syn (either sc or sp) conformation with respect to the $a$-amino group for class I aaRSs. In contrast, the attacking oxygen atom of the substrate AA has anti (either ap or ac) conformation with respect to the $a$-amino group in all class II aaRSs investigated.

Our quantum mechanical calculation of the transition states in GlnRS and HisRS within the respective active sites confirms that although the reactions in both cases progress through a common in-line displacement mechanism via a penta-coordinated transition state, the syn oxygen atom of the carboxylic group attacks the $aP$ of ATP in GlnRS (class I) and in contrast the anti oxygen atom attacks in the case of HisRS (class II). The schematic representations of the transition state geometries of GlnRS and HisRS computed at the ONIOM(HF/6–31G*: PM3) level of theory are shown in Figure 5(a) and (b), respectively. The transition states are characterized by imaginary frequency. The transition state geometry and its comparison with the reactant state geometry show that different set of residues are involved in the two aaRSs. The involvement of different set of residues in the two cases is related with the syn attack in the case of GlnRS and anti attack in HisRS. The analysis of the interactions between the active site residues and substrates in the reactant state and transition state are presented later.

To understand the unprecedented difference in the mode of nucleophilic attack by the substrate AA, we analyzed the electrostatic potential at molecular surfaces (ESP) of the substrates in absence of active site residues as present in the active site of the respective aaRSs. Computations of electrostatic potential of the substrates in two enzymes show that the difference in the spatial arrangement of ATP relative to the AAs between the two classes of aaRSs is responsible for this difference in mode of attack. Explicitly, the spatial arrangement of the triphosphate group of ATP with respect to the AA in class I and class II aaRSs is strikingly different. An imaginary plane is considered which is almost parallel to the sugar ring of ATP and nearly perpendicular to the adenosine base as well as passing through the $O_5$ atom of sugar ring. The carboxylic acid group of the substrate AA lies perpendicular to the imaginary plane and the amino group remains above the plane for both aaRSs. Note that the computation of ESP has no bearing on the choice of the imaginary plane, the later plane being only a guide for the eye. The carboxylic acid group of the AA is perpendicular to the plane shown in Figure 6(a) and (b), respectively and the amino group remains above the plane for all aaRSs. Although the orientations of AAs relative to the plane are same in all aaRSs in Figure 6(a) and (b), the orientations of the ATP are opposite in class I and class II aaRSs. The triphosphate group of ATP in class I aaRSs is above the plane (as shown in Figure 6(a)) while the same is below the plane in the case of class II aaRSs (as shown in Figure 6(b)). Since both the triphosphate group of ATP and carboxylic acid group of AA contains negative charges, it is favorable for the syn oxygen atom to be proximal with $aP$ and the anti oxygen atom is directed away from the charge distribution of ATP in class I aaRSs to minimize the repulsive interaction. In contrast, the orientation of ATP being exactly opposite in class II aaRSs, it is favorable for the anti oxygen atom to be proximal with $aP$ and the syn oxygen atom remains away from the charge distribution of ATP. Thus, our ESP analysis shows that the different
modes of attack in two classes of aaRSs are due to the difference in the mutual arrangements of ATP with respect to AAs in the active site of respective aaRSs.

We now proceed to show that the difference in the arrangements of the substrates in two aaRSs is coupled with the dissimilar active site organizations of two classes of aaRSs as exemplified by the studies in GlnRS and HisRS, respectively. Our calculation of the reaction mechanism by transition state analysis conclusively showed that residues such as A76 of tRNA, Lys270, and His43 are located in the close vicinity of the region of nucleophilic attack in GlnRS. In contrast, Arg259, Arg113, and Gln127 are located around the substrates in HisRS. The differences in the structural composition of residues are related with the different mode of attack in two cases. The organization of residues or ions and resulting interaction pattern hold the substrate in their respective mutual arrangements during the course of reaction. It is necessary to quantify the interactions between the substrates and active site residues which might facilitate the reaction. The influence of the active site residues on the reaction mechanism is quantitatively investigated in two ways. First, the interactions between the residues and substrates in the two aaRSs are measured by the computations of the electron densities at bond critical points between active site residues or ions and substrates. Secondly, we analyzed the influence of the active site residues and ions on the propensity of nucleophilic attack in the aaRSs using \( \delta q \).

In order to explore the role of active site residues in the nucleophilic attack in two representative aaRSs (GlnRS for class I and HisRS for class II), we computed the electron density at BCP between active site residues or ions and substrates in GlnRS and HisRS for reactant state as well as transition state. The changes in BCP values (Table 4 in the supplementary material) while going from the reactant state to the transition state quantitatively show the pattern of interaction driving the nucleophilic reaction. In the reactant state of GlnRS, A76 forms BCP with the substrate Gln only. The same residue forms BCP with both Gln and ATP in the transition state. Thus, A76 shifts from the proximity of Gln towards the reaction center to anchor Gln and ATP. Analogous role is performed by Arg113 and Arg259 in the case of HisRS. Both residues have BCP with ATP in reactant state and develop BCP with both substrates (His and ATP) in the transition state. The geometry of the transition state shows that in addition to A76, Lys270 and His43 develop BCPs in GlnRS. Similarly, Arg113, Gln127, and Arg259 form BCP in HisRS in the transition state. Most importantly, the arrangement of A76, Lys270, and His43 in GlnRS perfectly complements the spatial arrangement of atoms of the substrates during the attack by \( \text{syn} \) oxygen. The same arrangement of A76, Lys270, and His43 is not at all complementary to

the spatial arrangement of atoms of the substrates during the attack by \( \text{anti} \) oxygen in HisRS. The \( \text{anti} \) oxygen attack in HisRS is supported by pattern of interaction created by Arg113, Gln127, and Arg259. It remained a paradox that the different catalytic residues are present near the location of the nucleophilic attack in GlnRS and HisRS, respectively. Our result shows that the different set of residues in GlnRS and HisRS complement the spatial arrangements of the substrates in the respective transition states which are related with the nucleophilic attack by \( \text{syn} \) and \( \text{anti} \) oxygen in the respective aaRSs.

The composition of the active site residues near the reaction center varies from one aaRS to the other. This is puzzling since the chemical structures of the respective substrate like AA differ only in the side chain parts. Despite the fact that both groups are away from the reaction center and the chemical structures of the substrates at the reaction center being identical in both aaRS, unexpectedly the residues present in the first shell of active site of two aaRSs are different. It is a paradox that the molecular organization of active sites of both classes largely differ especially near the reaction center (location of the nucleophilic attack between the carboxylic acid group of AA and the \( \alpha \)-phosphorous atom of ATP, denoted as \( \alpha P \)). The importance of studying the molecular details of the reaction within active site is of major interest to understand the aminoacylation reaction within its biological perspective. Our analysis of the transition state in GlnRS and HisRS indicates that the molecular organization of the active sites in respective aaRSs complement the difference in spatial arrangement of atoms of substrates in each case and thereby facilitates the reaction. However, such analysis is yet to be carried out in other aaRSs and expected to reveal the origin of the variation of the active site organization from one aaRS to the other. This unresolved problem provides impetus for future studies about the correlation between active site organization and reaction therein.

The influence of the active site residues on the nucleophilic attack is also analyzed in terms of the \( \delta q \). First, the \( \delta q \) is computed when substrates are present only. This is shown in column A of the Table 1. The \( \delta q \) values enhance significantly for both class I and class II aaRSs when the active site residues close to the region of nucleophilic attack are incorporated. The resulting \( \delta q \) values are shown in column B in Table 1. The enhancement of \( \delta q \) implies that the propensity of the nucleophilic attack is facilitated by the presence of the added residues or ions. The comparison of the enhanced value of \( \delta q \) obtained by adding all residues and ions near the reaction center and the \( \delta q \) obtained in absence of all residues or ions are shown in Figure 7. The result indicates that the active site residues and ions reduce the unfavorable potential to facilitate the nucleophilic attack. It may be noted that the mode of nucleophilic attack and the active
site geometries are greatly complementary in the sense that their relative position is vital to the enhancement of $\delta q$. The observed influences of the active site residues or ions on $\delta q$ have the same trend using MPA and NPA schemes. A recent quantum chemical modeling study of Glycyl adenylate (Adrian-Scotto & Vasilescu, 2008) showed that the conformation of glycyl adenylate is stabilized by the electrostatic interactions with ionic residues and the $\text{Mg}^{2+}$ inside the active site and reduction of electrostatic potential of the phosphate group by the $\text{Mg}^{2+}$ ion and cationic Arg is suggested. This proposal corroborates our result from the charge population calculation that the active site residues and ions stabilize the substrates and facilitates the nucleophilic reaction in various aaRSs such as GlnRS, GluRS, TrpRS, TyrRS, LysRS, ProRS, and HisRS.

In principle, a limitation of the present study is that the complete macromolecular structure of the enzyme is not considered in the transition state calculation or calculation of the bond critical points using AIM theory and only first-shell neighbors are considered. Since the result of the present paper is primarily dependent on the residues or ions in the immediate vicinity of the substrate (due to the $1/r^6$ dependence of the interactions therein), the effect of second-shell neighbors and beyond that is less significant. This can be confirmed from the computational study where the complete enzyme structure is considered comparing the results from the smaller quantum mechanical models with larger models (Dutta Banik & Nandi, 2011). However, the incorporation of complete macromolecular structure is desirable considering the hydrophobic environment of the enzymatic active site structure and its influence on the reactions therein (Kahn & Bruice, 2003). Hypothetically, the reaction between AA and ATP leading to the formation of adenylate in bulk water is not only dependent on the electrostatic interaction between the two species, but also on the hydrophobic interaction (the process required to bring two solutes from infinity to a separation to a final configuration within a solvent at constant temperature and pressure) (Ben Naim, 1992; Pratt, 1985; Tanford, 1980). Similarly, the same reaction in enzyme active site is dependent on the electrostatic interaction between the two species in the environment of active site but also on the solvophobic interaction (Yaacobi & Ben Naim, 1974) therein when the active site is approximated by a non-aqueous solvent medium. The efficiency of the reaction in the enzyme over the aqueous medium is dependent on the how favorable in later compared to former case.

Due to the syn and anti nucleophilic attacks in class I and class II, respectively the conformations of the product adenylate is different for class I and class II aaRSs (also noted in the present work from crystal structures as shown in supplementary material). This might has an implication in facilitating the second step (tRNA charging). The A76 (adenosine end) of tRNA occupy different sides of adenylate in class I (proximal to the syn oxygen) and class II (proximal to the anti oxygen) aaRSs. A76 approach the carboxyl group of the adenylate via 2$\text{OH}$ group in case of class I aaRSs and via 3$\text{OH}$ group in case of class II aaRSs. One of the free oxygen atom attached with the $\alpha P$ of the aminoacyl adenylate acts as proton attractor from the hydroxyl group. In order to accept the proton, the $\alpha P$ needs to be proximal with the hydroxyl group of tRNA. The $\alpha P$ being attached with the syn oxygen in class I aaRS (an outcome of syn attack in the activation step) can easily accept the proton attached with the proximal OH group of tRNA. In contrast, the $\alpha P$ attached with the anti oxygen (which is a result of the anti attack) can accept the proton attached with the proximal OH group of tRNA. Thus, the syn/anti attack in two aaRS discovered in this work is expected to have a greater significance in the aminoacylation process. The hitherto unknown difference in the reaction mechanism between two class of aaRSs at the molecular level have implication in the understanding of the organization of the active site structure of the enzyme as the active site is the closest vicinity of the reactants as mentioned in the introduction. The difference in the mode of nucleophilic attack at molecular level observed in the present paper might have implication in the observed variation in the structure of the active site, particularly near the reaction center and is worth investigating. This is the subject of future study. Such understanding may be utilized to develop novel enzyme-mimetic systems and understanding the diverse roles of aaRSs beyond aminoacylation (Kim, You, & Hwang, 2011).

**Concluding remarks**

The present work reveals a novel difference in the mode of inline attack at the molecular level by the oxygen atom of the carboxylic group of substrate AA to the $\alpha P$ atom of ATP in the common scheme of nucleophilic reaction of the activation step of aminoacylation reaction between class I (GluRS, GlnRS, TyrRS, TrpRS, LeuRS, ValRS, IleRS, CysRS, and MetRS) and class II (HisRS, LysRS, ProRS, AspRS, AsnRS, AlaRS, GlyRS, PheRS, and ThrRS) aaRSs for the first time. The syn oxygen atom of the carboxylic group attacks the $\alpha P$ of ATP in class I aaRSs, while the anti oxygen atom attacks in the case of class II aaRSs. The relative arrangement of the negative charge distribution of the oxygen atoms of the ATP and that of the carboxylic acid group of AA is directed in opposite directions in class I and class II aaRSs. The dissimilar relative orientations of the AA and ATP in the two classes of aaRSs and the concomitant variations in ESP result in the observed different modes of attack in the two cases. It is favorable for the syn oxygen atom to be proximal with $\alpha P$ and to act as the
attacking atom. Consequently, the corresponding anti oxygen atom can be directed away from the charge distribution of ATP in class I aaRSs to minimize the repulsive interaction. In contrast, the orientation of ATP being exactly opposite in class II aaRSs, it is favorable for anti oxygen atom to be proximal with αP acts as the attacking atom and the syn oxygen atom remain away from the charge distribution of ATP. This is confirmed from the variation of the strength of the interaction between oxygen atoms attached with the carboxylic acid and αP of ATP as a function of variation of the conformation of oxygen. The maximum ρb at the syn conformation for class I aaRSs indicates that it is more probable for the syn oxygen atom to attack the αP atom to form covalent bond. In contrast, the ρb is maximum at the anti conformation for class II aaRSs indicating the attack by anti oxygen atom is more probable and can form covalent bond with the αP atom in the adenylate state. The study of the variation of interaction energy as a function of separation between a pair of oxygen atoms of the carboxylic acid group of substrate AA and αP atom of ATP in the reactant state for class I and class II aaRSs confirms that the approach of the oxygen with syn (either sc or sp) conformation is favorable for class I aaRSs (except TprRS) and approach by the anti (either ap or ac) conformation oxygen atom is favorable in all class II aaRSs. A comparative study of the reaction mechanisms of the activation step in a class I aaRS (Glutaminyl tRNA synthetase) and in a class II aaRS (Histidyl tRNA synthetase) is carried out by the transition state analysis. The AIM analysis of the interaction between active site residues or ions and substrates is carried out in the reactant state and the transition state. The result shows that the observed novel difference in the mechanism is correlated with the organizations of the active sites of the respective aaRSs. The result has implication in understanding the experimentally observed different modes of tRNA binding in the two classes of aaRSs.

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