# 博士論文審査報告書

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|---|---|
| 氏名 | 坂部　翔 |
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| 論 文 題 目 | Theoretical study of catalytic mechanisms of editing reaction by aminoacyl-tRNA synthetases<br>「アミノアシル tRNA 合成酵素によるエディティング反応における触媒メカニズムの理論解析」 |
| 論文審査委員 | （主査）　教授　水島 恒裕 |
| | （副査 ）　教授　吉久 徹 |
| | （副査）　教授　舘野 賢 |
| | （副査）　教授　高井 和幸<br>（愛媛大学大学院理工学研究科，物質生命工学専攻応用科学講座） |
| | （副査）　教授　Thomas Simonson<br>(Biochemistry Laboratory, Biology Department, Ecole Polytechnique,) |

*Simonson 委員の審査結果については別紙（英文）として添付する。

1. 論文内容の要旨

アミノアシル tRNA 合成酵素 (aaRS) は，特異的なアミノ酸を tRNA に付加する転移反応（aminoacylation）を触媒する酵素である。立体構造が互いに良く似たアミノ酸もあるため，非特異的なアミノ酸を tRNA に結合する aaRS も存在し（mis-aminoacylation），それらの aaRS は誤ったアミノ酸転移を校正するための加水分解反応も触媒する（エディティング反応）。先行する理論研究により（所属する研究室による），ロイシンの酵素（LeuRS）と valyl-$tRNA^{Leu}$ との複合体の立体構造が構築され、求核剤（水分子）が同定された。さらに求核剤を活性化するシッフ塩基が，タンパク質（LeuRS）部分には無く，$tRNA^{Leu}$ 自身の 3' 末端アデノシン 76（A76）における O3'（リボース）であることも明らかになった。これは，エディティングがリボザイム反応であることを意味すると同時に，タンパク質部分（LeuRS）は高エネルギ状態の形成を抑える役割を果たしており，両者によるハイブリッド触媒であることが分かった。実際 A76 の O3' を欠損させた $tRNA^{Leu}$ では，活性が $10^4$ 倍も低下することが，生化学実験によって最近，明らかになった。

バリンの酵素（ValRS）は，LeuRS と極めて近縁であることから，立体構造が非常に良く似ているために，双方とも同様の反応メカニズムであると期待される。ところが Val の系では，A76 の O3' を欠損させた $tRNA^{Val}$ 変異体においても，活性の低下はわずかであり（~10 倍），その理由は長い間，謎であった。実験結果におけるこの矛盾の原因とそのメカニズムを解明するために，X 線結晶構造解析により ValRS•$tRNA^{Val}$ 複合体の立体構造が決定されたが，活性中心の存在するエディティング（CP1）ドメインについては，ドメイン自体の回転運動のために，立体構

造の正確な決定には至らなかった。そのため CP1 ドメイン単独での立体構造が独立に決定されたが，その他の部位との接続部等の立体構造は依然不明であり，したがってそれら不明の箇所と $tRNA^{Val}$ との正確な相互作用もまた未知である。

そこで本研究では，ValRS と threonyl-$tRNA^{Val}$ の複合体の立体構造を理論的に構築し、これを用いて分子動力学（MD）計算を実行することにより，完全に水和した ValRS•threonyl-$tRNA^{Val}$ 複合体の立体構造を得た。得られた複合体の溶液構造を用いて，エディティング反応（初期）のメカニズムを解析した結果，求核剤（水分子）が同定され，Val の系でも同様にハイブリッド触媒メカニズムが作用していることを見出した。そこでさらに複合体の溶液構造において，水和（水分子の配置）と水素結合ネットワークを解析した結果，A76 の O3' を欠損させた $tRNA^{Val}$ においては，代わりにタンパク質（ValRS）の Asp 残基が，O3' に代わって求核剤を活性化し得ることが示唆された。すなわち Val の系（ValRS•threonyl-$tRNA^{Val}$ 複合体）では，リボザイム（$tRNA^{Val}$）の活性化部位の欠損が，ハイブリッド触媒（O3'）からタンパク質酵素（Asp 残基）への遷移によって補償され得ることを意味している。これは，生命の起源やその後の分子進化においても，重要な意味を有する遷移であると考えられる。

さらに本研究では，エディティング反応の進行に伴う，活性中心の電子構造の変化を追跡した。その結果，反応に直接寄与する分子軌道（MO）（求核剤である水分子の酸素原子の *2p* 軌道）が，反応の初期においては HOMO よりもはるかにエネルギレベルの低い状態に位置しており，求核反応の進行に伴って，この MO のエネルギレベルが次第に上昇し，HOMO に接近することが分かった。このようにして，反応の初期においてはエネルギレベルが HOMO から大きく離れていた活性（Reactive）な MO が，反応の進行に伴い次第に上昇し，LUMO と混成することにより，遂に求核反応が生じると考えられる。実際この描像もまた，Leu の系では既に所属する研究室において明らかにされている。よって Val の系においても，同様な電子ダイナミクスが反応メカニズムを制御するものと考えられる。このようにして，生体システムに特有な，特徴的な事象を見出すと共に，さらに Leu と Val の系の反応メカニズムが，生化学実験データに基づく従来の議論と異なり，本質的に同じであることをこれらの結果は意味するものである。

2. 論文審査結果

本研究の解析対象である aaRS は，Leu の系におけるエディティング反応のメカニズムが既に理論的に解析され，リボザイムとタンパク質によるハイブリッド触媒であることが，*ab initio* 電子状態計算と古典場，および MD 計算の３者を組合せた，先端的な解析法により先行して明らかになっている。その近縁である Val の酵素（ValRS）は，アミノ酸配列や立体構造が極めてよく保存されていることから，そのエディティング反応は同様にハブリッド触媒メカニズムに拠るものであることが期待される。ところが生化学実験によれば，リボザイムの活性中心（A76 の O3'）を除去しても，Val の系における酵素活性は $\sim 10^{-1}$ 程度しか低下せず，その理由が長い間，謎として残され，先の Leu の系（$\sim 10^{-4}$ に低下）とは根本的に異なる反応メカニズムであると考えられた。本研究は，生命の根幹に関わるこれらの謎を，電子構造に基づく本質的な理解を得ることを目指して挑んだものであり，国際的にも極めて高い水準にある。このように本研究は，実験データに内在する矛盾の理由を，構造モデリング技術，MD 計算および *ab initio* 電子状態計算などを組合せることにより，先端的な理論解析技術を構築・駆使して解明し，今後さらに実験・理論の両面で，反応メカニズムの全貌の解明に向けた飛躍的な発展の基礎となり得る重要な成果であると評価できる。

よって，本論文は博士（理学）の学位論文として価値のあるものと認める。

また，平成 31 年 1 月 17 日，論文内容およびこれに関連する事項について試問を行った結果，合格と判定した。

# Evaluation Report for Doctoral Thesis

Title : Theoretical study of catalytic mechanisms of editing reaction by aminoacyl-tRNA synthetases

Applicant : Kakeru Sakabe

1. Abstract of the thesis

Aminoacyl-tRNA synthetases (aaRSs) are protein enzymes that attach the cognate amino acids to tRNAs. aaRSs are classified into two classes, classes I and II, each of which is further classified into three subclasses, a, b, and c, on the basis of the amino acid sequences and three-dimensional (3D) structures. Some aaRSs mis-attach non-cognate amino acids to tRNA (mis-aminoacylation) due to the similarities of the chemical structures of some amino acids. In such cases, those aaRSs catalyze the hydrolysis for proofreading of the mis-aminoacylation (editing reaction). In the previous theoretical studies performed in our lab, a fully-hydrated structural model of the complex of leucyl-tRNA synthetase (LeuRS) (class Ia) and (mis-aminoacylated) valyl-$tRNA^{Leu}$ was built, and the nucleophilic water molecule was identified in the catalytic center. Moreover, the activator of the nucleophile acting as the Schiff base was identified to be O3' of adenosine76 (A76), which is the 3' terminal nucleotide of $tRNA^{Leu}$, and thus revealed that the key factor of the catalysis existed in the tRNA moiety.

Accordingly, this catalytic system is ribozymal, operating with the protein, and so was referred to as the hybrid ribozyme/protein catalyst. In fact, very recently, biochemical experiments have shown that the defect of O3' atom of A76 to H3' reduced the activity of the editing reaction catalyzed by the LeuRS•valyl-$tRNA^{Leu}$ complex, by ~$10^4$-fold. By contrast, in the Val system (class Ia), effects of the similar defect in $tRNA^{Val}$ (i.e., the replacement of O3' atom of A76 with H3') were marginal in the activity of the editing catalyzed by the ValRS•threonyl-$tRNA^{Val}$ complex (the rate reduction was measured to be ~10-fold). This was too strange to understand the reaction mechanisms, since both Leu and Val systems are closely related (class Ia). Actually, for LeuRS and ValRS, the amino acid sequences and three-dimensional (3D) structures are strikingly similar.

In order to understand this experimental discrepancy, Mr. Sakabe built a structural model of the ValRS•threonyl-$tRNA^{Val}$ complex in the thesis. In the crystal structure of the ValRS•$tRNA^{Val}$ complex, the 3D structure of the editing domain (CP1 domain) moiety was not determined because of the rotational motions of the domain. The crystallographers analyzed the isolated CP1 domain and determined the crystal structure. However, the 3D structures of the moieties connecting the CP1 domain and the remaining main body of ValRS, and the interactions between $tRNA^{Val}$ and those unknown structures were not elucidated, although the analysis of the catalytic mechanisms required those 3D

structures. Thus, Mr. Sakabe employed these two crystal structures to build the structural model of the ValRS•threonyl-tRNA$^{Val}$ complex, where mis-aminoacylated tRNA$^{Val}$ was involved. In order to obtain the fully-solvated solution structure, he performed molecular dynamics (MD) simulations starting from the modeled structure of the ValRS•threonyl-tRNA$^{Val}$ complex.

As a result of the analysis, the nucleophilic water molecule was identified, which thus suggested that the editing reaction of the ValRS•threonyl-tRNA$^{Val}$ complex was operated in a manner similar to the Leu system, i.e., the hybrid ribozyme/protein catalyst mechanism. Moreover, he also analyzed the hydration of the catalytic site in the structural model (i.e., the locations of water molecules and hydrogen bond networks), and thus anticipated that an Asp residue of ValRS could compensate the role of O3' of A76 as the Schiff base; i.e., the amino acid residue can activate the nucleophilic water, even when O3' of A76 is replaced with H3'. This proposal means that the loss of the ribozymal reactive factor can be replaced through the transition from the hybrid catalyst toward the protein enzyme in the ValRS•threonyl-tRNA$^{Val}$ complex. This also could occur in the evolutional processes and the origin of life, i.e., the transition from the RNA world to RNP world.

Furthermore, he analyzed the electronic structure of the catalytic center in the initial stages of the editing reaction, employing hybrid *ab initio* quantum mechanics (QM)/molecular mechanics (MM) calculations. As a result of this analysis, he found that in the initial structure of the reaction, the reactive MO that was responsible for the reaction (i.e., *2p* orbital of O atom in the nucleophilic water) was located in a significantly lower energy level than that of the HOMO. However, as the reaction proceeded, the energy level of the MO increased toward that of the HOMO. Thus, the energy level of the reactive MO could be elevated toward that of the HOMO, and then hybridize with the LUMO, which is corresponding to the bond formation in the editing reaction. This picture has already elucidated in the Leu system, and thus the Val system would be identical to the Leu system in the catalytic mechanisms of the editing reaction.

2. Evaluation of the thesis and the final examination

The editing function for mis-aminoacylated tRNAs is crucial to ensure the fidelity of translational system, and the catalytic mechanisms were resolved theoretically for the Leu system in previous studies; i.e., *ab initio* electronic structure calculations coupled with the classical field representation revealed that the editing was ribozymal together with the protein (LeuRS) moiety cooperating so as to hinder the high energy states in the catalysis. Also, mechanism of the Val system was supposed to be identical, since both Leu and Val systems are closely related as class Ia aaRSs (i.e., the highly conserved amino acid sequences and 3D structures). However, for the Val system, previous biochemical experiments showed that the defect of the ribozymal activator in tRNA$^{Val}$ marginally reduced the rate of reaction (~1/10), and thus the enzymatic mechanisms of the Val system were anticipated to be completely different from the Leu system. Thus, the reason of these critical experimental contradictions was remained to be unknown for a long time.

This thesis is aimed to provide substantial understandings of this problem based on evaluations of the electronic structures in the catalytic centers. For this purpose, a fully-solvated, atomistic solution structure of ValRS in the complex with mis-aminoacylated $tRNA^{Val}$ was built by combining the two crystal structures of the ValRS•$tRNA^{Val}$ complex and the isolated editing domain. The MD simulations identified the nucleophilic water, which suggested that the enzymatic mechanisms were identical to the Leu system. Also, the *ab initio* electronic structure calculations showed that the reactive MOs were elevated as the reaction proceeded, which suggested the mechanisms similar to the Leu system again.

As a result, the analysis indicated that the Val mechanisms were identical to the Leu mechanisms. The structural analysis of the complex of ValRS and mis-aminoacylated $tRNA^{Val}$ further suggested that for the mutant of mis-aminoacylated $tRNA^{Val}$, an Asp residue, instead of O3' of A76 in the tRNA, can act as the Schiff base, which means that the hybrid catalyst is replaced with a protein enzyme. This is a novel proposal to explain the experimental discrepancy in the Leu and Val systems. Furthermore, this scenario would have been adopted for the transition from the RNA to RNP worlds in the origin of life.

Thus, the thesis completed in fulfillment of the requirements for the degree of Doctor of Science in the Graduate School of Life Science.

The committee also certifies that the applicant passed the final oral examination on his thesis and related issues held on January 17 in 2019.

The chief examiner : Tsunehiro Mizushima

The second readers : Toru Yoshihisa

: Masaru Tateno

: Kazuyuki Takai

(Graduate School of Science and Engineering, Ehime University, Professor)

: Thomas Simonson

(Ecole Polytechnique, Biology Department, Biochemistry Laboratory, Professor)

# 博士論文審査報告書

論 文 題 目：Theoretical study of catalytic mechanisms of editing reaction by aminoacyl-tRNA synthetases
「アミノアシル tRNA 合成酵素によるエディティング反応における触媒メカニズムの理論解析」

申請者：坂部　翔

1. 論文内容の要旨

アミノアシル tRNA 合成酵素 (aaRS) は，特異的なアミノ酸を tRNA に付加する転移反応 (aminoacylation) を触媒する酵素である。立体構造が互いに良く似たアミノ酸もあるため，非特異的なアミノ酸を tRNA に結合する aaRS も存在し (mis-aminoacylation)，それらの aaRS は誤ったアミノ酸転移を校正するための加水分解反応も触媒する (エディティング反応)。先行する理論研究により (所属する研究室に拠る)，ロイシンの酵素 (LeuRS) と valyl-$tRNA^{Leu}$ との複合体の立体構造が構築され、求核剤 (水分子) が同定された。さらに求核剤を活性化するシッフ塩基が，タンパク質 (LeuRS) 部分には無く，$tRNA^{Leu}$ 自身の 3' 末端アデノシン 76 (A76) における O3' (リボース) であることも明らかになった。これは，エディティングがリボザイム反応であることを意味すると同時に，タンパク質部分 (LeuRS) は高エネルギ状態の形成を抑える役割を果たしており，両者によるハイブリッド触媒であることが分かった。実際 A76 の O3' を欠損させた $tRNA^{Leu}$ では，活性が $10^4$ 倍も低下することが，生化学実験によって最近，明らかになった。

バリンの酵素 (ValRS) は，LeuRS と極めて近縁であることから，立体構造が非常に良く似ているために，双方とも同様の反応メカニズムであると期待される。ところが Val の系では，A76 の O3' を欠損させた $tRNA^{Val}$ 変異体においても，活性の低下はわずかであり (~10 倍)，その理由は長い間，謎のままであった。実験結果におけるこの矛盾の原因とメカニズムを解明するために，X 線結晶構造解析により ValRS•$tRNA^{Val}$ 複合体の立体構造が決定されたが，活性中心の存在するエディティング (CP1) ドメインについては，ドメイン自体の回転運動のために，その立体構造の正確な決定には至らなかった。そのため CP1 ドメイン単独での立体構造が独立に決定された。しかし，その他の部位との接続部等の立体構造は依然不明であり，したがってそれら不明の箇所と $tRNA^{Val}$ との相互作用もまた未知である。

そこで本研究では，ValRS と threonyl-$tRNA^{Val}$ の複合体の立体構造を理論的に構築し、これを用いて分子動力学 (MD) 計算を実行することにより，完全に水和した ValRS•threonyl-$tRNA^{Val}$ 複合体の立体構造を得た。得られた複合体の溶液構造を用いて，エディティング反応 (初期) のメカニズムを解析した結果，求核剤 (水分子) が同定され，Val の系でも同様にハイブリッド触媒メカニズムが作用していることを見出した。そこでさらに複合体の溶液構造において，水和 (水分子の配置) と水素結合ネットワークを解析した結果，A76 の O3' を欠損させた $tRNA^{Val}$ においては，代わりにタンパク質 (ValRS) の Asp 残基が，O3' に代わって求核剤を活性化し得ることが分かった。すなわち Val の系 (ValRS•threonyl-$tRNA^{Val}$ 複合体) では，リボザイム ($tRNA^{Val}$) の活性化部位の欠損が，ハイブリッド触媒 (O3') からタンパク質酵素 (Asp 残基) への遷移によって補償され得ることを意味している。これは，生命の起源やその後の分子進化においても，重要な意味を有する遷移であると考えられる。

さらに本研究では，エディティング反応の進行に伴う，活性中心の電子構造の変化を追跡した。その結果，反応に直接寄与する分子軌道（MO）（求核剤である水分子の酸素原子の *2p* 軌道）が，反応の初期においては HOMO よりもはるかにエネルギレベルの低い状態に位置しており，求核反応の進行に伴って，この MO のエネルギレベルが次第に上昇し，HOMO に接近することが分かった。このようにして，反応の初期においてはエネルギレベルが HOMO から大きく離れていた活性（Reactive）な MO が，反応の進行に伴い次第に上昇し，LUMO と混成することにより，遂に求核反応が生じると考えられる。実際この描像は，Leu の系では既に本研究室において明らかにされている。よって Val の系においても，同様な電子ダイナミクスが反応メカニズムを制御するものと考えられ，生体システムに特徴的な事象を見出すと共に，Leu と Val の系の反応メカニズムが，従来の議論と異なり，本質的に同じであることを意味するものである。

2. 論文審査結果

本研究の解析対象である aaRS は，Leu の系におけるエディティング反応のメカニズムが既に理論的に解析され，リボザイムとタンパク質によるハイブリッド触媒であることが，*ab initio* 電子状態計算と古典場，および MD 計算の３者を組合せた，先端的な解析法により先行して明らかになっている。その近縁である Val の酵素（ValRS）は，アミノ酸配列や立体構造が極めてよく保存されていることから，エディティング反応は同様にハブリッド触媒メカニズムに拠るものであることが強く期待される。ところが生化学実験によれば，リボザイムの活性中心（A76 の O3'）を除去しても，Val の系における酵素活性は $\sim 10^{-1}$ 程度しか低下せず，先の Leu の系（$\sim 10^{-4}$ に低下）とは根本的に異なる反応メカニズムであると考えられ，長い間，謎として残されていた。本研究は，生命の根幹に関わるこれらの謎を，電子構造に基づいて本質的な理解を得ることを目指して達成したものであり，国際的にも高い水準にある研究といえる。このように本研究は，実験データに内在する矛盾の理由を，構造モデリング技術，MD 計算および *ab initio* 電子状態計算などを組合せることにより，先端的な理論解析技術を構築・駆使して解明し，今後さらに実験・理論の両面で，反応メカニズムの全貌の解明に向けた飛躍的な発展の基礎となり得る重要な成果であると評価できる。

よって，本論文は博士（理学）の学位論文として価値のあるものと認める。

また，平成 31 年 1 月 17 日，論文内容およびこれに関連する事項について試問を行った結果，合格と判定した。

主査 ： 水島 恒裕　　印

副査 ： 吉久 徹　　印

： 舘野 賢　　印

： 高井 和幸　　印

（愛媛大学大学院理工学研究科，物質生命工学専攻応用科学講座教授）

： Thomas Simonson　署名

(Biochemistry Laboratory, Biology Department, Ecole Polytechnique, Professor)

*Simonson 委員の審査結果については別紙（英文）として添付する。

# Evaluation Report for Doctoral Thesis

Title : Theoretical study of catalytic mechanisms of editing reaction by aminoacyl-tRNA synthetases

Applicant : Kakeru Sakabe

1. Abstract of the thesis

Aminoacyl-tRNA synthetases (aaRSs) are protein enzymes that attach the cognate amino acids to tRNAs. aaRSs are classified into two classes, classes I and II, each of which is further classified into three subclasses, a, b, and c, on the basis of the amino acid sequences and three-dimensional (3D) structures. Some aaRSs mis-attach non-cognate amino acids to tRNA (mis-aminoacylation) due to the similarities of the chemical structures of some amino acids. In such cases, those aaRSs catalyze the hydrolysis for proofreading of the mis-aminoacylation (editing reaction). In the previous theoretical studies performed in our lab, a fully-hydrated structural model of the complex of leucyl-tRNA synthetase (LeuRS) (class Ia) and (mis-aminoacylated) valyl-$tRNA^{Leu}$ was built, and the nucleophilic water molecule was identified in the catalytic center. Moreover, the activator of the nucleophile acting as the Schiff base was identified to be O3' of adenosine76 (A76), which is the 3' terminal nucleotide of $tRNA^{Leu}$, and thus revealed that the key factor of the catalysis existed in the tRNA moiety.

Accordingly, this catalytic system is ribozymal, operating with the protein, and so was referred to as the hybrid ribozyme/protein catalyst. In fact, very recently, biochemical experiments have shown that the defect of O3' atom of A76 to H3' reduced the activity of the editing reaction catalyzed by the LeuRS•valyl-$tRNA^{Leu}$ complex, by ~$10^4$-fold. By contrast, in the Val system (class Ia), effects of the similar defect in $tRNA^{Val}$ (i.e., the replacement of O3' atom of A76 with H3') were marginal in the activity of the editing catalyzed by the ValRS•threonyl-$tRNA^{Val}$ complex (the rate reduction was measured to be ~10-fold). This was too strange to understand the reaction mechanisms, since both Leu and Val systems are closely related (class Ia). Actually, for LeuRS and ValRS, the amino acid sequences and three-dimensional (3D) structures are strikingly similar.

In order to understand this experimental discrepancy, Mr. Sakabe built a structural model of the ValRS•threonyl-$tRNA^{Val}$ complex in the thesis. In the crystal structure of the ValRS•$tRNA^{Val}$ complex, the 3D structure of the editing domain (CP1 domain) moiety was not determined because of the rotational motions of the domain. The

crystallographers analyzed the isolated CP1 domain and determined the crystal structure. However, the 3D structures of the moieties connecting the CP1 domain and the remaining main body of ValRS, and the interactions between $tRNA^{Val}$ and those unknown structures were not elucidated, although the analysis of the catalytic mechanisms required those 3D structures. Thus, Mr. Sakabe employed these two crystal structures to build the structural model of the ValRS•threonyl-$tRNA^{Val}$ complex, where mis-aminoacylated $tRNA^{Val}$ was involved. In order to obtain the fully-solvated solution structure, he performed molecular dynamics (MD) simulations starting from the modeled structure of the ValRS•threonyl-$tRNA^{Val}$ complex.

As a result of the analysis, the nucleophilic water molecule was identified, which thus suggested that the editing reaction of the ValRS•threonyl-$tRNA^{Val}$ complex was operated in a manner similar to the Leu system, i.e., the hybrid ribozyme/protein catalyst mechanism. Moreover, he also analyzed the hydration of the catalytic site in the structural model (i.e., the locations of water molecules and hydrogen bond networks), and thus anticipated that an Asp residue of ValRS could compensate the role of O3' of A76 as the Schiff base; i.e., the amino acid residue can activate the nucleophilic water, even when O3' of A76 is replaced with H3'. This proposal means that the loss of the ribozymal reactive factor can be replaced through the transition from the hybrid catalyst toward the protein enzyme in the ValRS•threonyl-$tRNA^{Val}$ complex. This also could occur in the evolutional processes and the origin of life, i.e., the transition from the RNA world to RNP world.

Furthermore, he analyzed the electronic structure of the catalytic center in the initial stages of the editing reaction, employing hybrid *ab initio* quantum mechanics (QM)/molecular mechanics (MM) calculations. As a result of this analysis, he found that in the initial structure of the reaction, the reactive MO that was responsible for the reaction (i.e., *2p* orbital of O atom in the nucleophilic water) was located in a significantly lower energy level than that of the HOMO. However, as the reaction proceeded, the energy level of the MO increased toward that of the HOMO. Thus, the energy level of the reactive MO could be elevated toward that of the HOMO, and then hybridize with the LUMO, which is corresponding to the bond formation in the editing reaction. This picture has already elucidated in the Leu system, and thus the Val system would be identical to the Leu system in the catalytic mechanisms of the editing reaction.

2. Evaluation of the thesis and the final examination

The editing function for mis-aminoacylated tRNAs is crucial to ensure the fidelity of translational system, and the catalytic mechanisms were resolved theoretically for the Leu system in previous studies; i.e., *ab initio* electronic structure calculations coupled with the classical field representation revealed that the editing was ribozymal together with the protein (LeuRS) moiety cooperating so as to hinder the high energy states in the catalysis. Also, mechanism of the Val system was supposed to be identical, since both Leu and Val systems are closely related as class Ia aaRSs (i.e., the highly conserved amino acid sequences and 3D structures). However, for the Val system,

previous biochemical experiments showed that the defect of the ribozymal activator in $tRNA^{Val}$ marginally reduced the rate of reaction (~1/10), and thus the enzymatic mechanisms of the Val system were anticipated to be completely different from the Leu system. Thus, the reason of these critical experimental contradictions was remained to be unknown for a long time.

This thesis is aimed to provide substantial understandings of this problem based on evaluations of the electronic structures in the catalytic centers. For this purpose, a fully-solvated, atomistic solution structure of ValRS in the complex with mis-aminoacylated $tRNA^{Val}$ was built by combining the two crystal structures of the ValRS•$tRNA^{Val}$ complex and the isolated editing domain. The MD simulations identified the nucleophilic water, which suggested that the enzymatic mechanisms were identical to the Leu system. Also, the *ab initio* electronic structure calculations showed that the reactive MOs were elevated as the reaction proceeded, which suggested the mechanisms similar to the Leu system again.

As a result, the analysis indicated that the Val mechanisms were identical to the Leu mechanisms. The structural analysis of the complex of ValRS and mis-aminoacylated $tRNA^{Val}$ further suggested that for the mutant of mis-aminoacylated $tRNA^{Val}$, an Asp residue, instead of O3' of A76 in the tRNA, can act as the Schiff base, which means that the hybrid catalyst is replaced with a protein enzyme. This is a novel proposal to explain the experimental discrepancy in the Leu and Val systems. Furthermore, this scenario would have been adopted for the transition from the RNA to RNP worlds in the origin of life.

Thus, the thesis completed in fulfillment of the requirements for the degree of Doctor of Science in the Graduate School of Life Science.

The committee also certifies that the applicant passed the final oral examination on his thesis and related issues held on January 17 in 2019.

The chief examiner : Tsunehiro Mizushima

The second readers : Tohru Yoshihisa

: Masaru Tateno

: Kazuyuki Takai

(Graduate School of Science and Engineering, Ehime University, Professor)

: Thomas Simonson

(Ecole Polytechnique, Biology Department, Biochemistry Laboratory, Professor)

# Report on the PhD manuscript of Kakeru Sakabe

K Sakabe presents a PhD manuscript that describes his work on the valyl-tRNA synthetase enzyme (ValRS) and its catalytic mechanism, using methods from computational chemistry. ValRS is one of 20 aminoacyl-tRNA synthetase enzymes (aaRSs) that each attach a specific amino acid to a specific tRNA. The tRNA carries a triplet of nucleotides that code for the specific amino acid, and the correspondence between the amino acid and tRNA establishes the genetic code. The aaRS enzyme family is thus very important for evolution and biology, and a precise understanding of the mechanism of these enzymes is an important problem in biochemistry.

In addition to tRNA aminoacylation, ValRS and several other aaRSs catalyze a second, editing reaction. There, a tRNA that has been accidentally ligated to the wrong amino acid is hydrolyzed, or "edited" to remove the amino acid. This reaction takes place in a second active site, which is distinct from the active site for tRNA aminoacylation. The detailed mechanism of both the primary aminocylation reaction and the editing reaction remains unknown for most aaRSs.

It is difficult to determine an enzyme's reaction mechanism by experiments alone. Indeed, the intermediate states along the pathway are unstable and short-lived, and cannot be directly captured by crystallography or nuclear magnestic resonance, for example. In contrast, molecular dynamics simulations allow one to directly visualize states along the reaction pathway. The information is only reliable if the simulation employs an accurate energy function, which treats the active site quantum mechanically and the rest of the system with classical or "molecular" mechanics. Such "QM/MM" models have made great progress in recent years, and can be used to perform very long simulations thanks to the computer power available today. The candidate's research group has extensive experience and expertise with these complex techniques, and has applied them to several other aaRS enzymes in the past. Thus, they showed that the related enzyme leucyl-tRNA synthetase (LeuRS) has an editing reaction mechanism where the tRNA substrate participates actively, activating a nucleophilic water. Here, the candidate describes his work on the ValRS editing reaction.

The thesis begins with an overview of aaRSs, their structure and function. The main relevant simulation methodology is described briefly. The main body of the thesis presents the ValRS work, and follows the format of a research article. Indeed, the work described here was published as an article in the Journal of the Physical Society of Japan in 2018. The first step was to produce a structural model of the enzyme, by combining experimental structures of two large fragments: the editing domain and the rest of the protein. The protein was modeled in a complex with the normal substrate tRNA(Val) and with two altered substrates: tRNA(Val) ligated to threonine (Thr), instead of valine, and a mutant tRNA(Val), ligated to threonine, where the terminal ribose group is in the

deoxy form with no O2' atom. Special care was taken to position correctly amino acids and nucleotides in the active site.

The next main task was to perform QM/MM simulations of the ValRS complex bound to tRNA(Val)–Thr (with and without its O2' atom). The tRNA was positioned in the editing active site, and the structure and motions of the active site groups, including water molecules, were carefully studied. The position and motions of those groups showed that the "normal" tRNA(Val)–Thr is hydrolyzed with the help of the terminal ribose and its O2' atom, which helps activate a nucleophilic water, which then attacks the labile bond. Since the tRNA participates directly in the reaction, the system can be considered a mixed protein-RNA enzyme. This was also shown earlier to be the case also for the homologous LeuRS enzyme and its editing reaction. In contrast, when a modified tRNA is involved, with no terminal O2' atom, the reaction proceeds almost as well, because there is room for a water molecule to approach the labile bond, occupying space that was otherwise taken by the O2'. Thus, with the modified tRNA, the enzyme does not use RNA groups directly in the catalytic mechanism: it functions as a protein enzyme.

The manuscript ends with a discussion, which considers the importance of the ValRS example for enzyme evolution. The author proposes that ValRS represents a transition between a mixed protein-RNA enzyme and an enzyme that uses only protein groups for catalysis. Thus, this ancient enzyme might have captured a major step in the transition from an early biosphere with mostly RNA enzymes and the modern biosphere with mostly protein enzymes. This is a significant result for our understanding of evolution.

Overall, the thesis represents an interesting body of work on a very important system. It has contributed to move the domain forward and increase our understanding of an important class of biocatalysts. The work has led to the publication of a high quality research article, with the candidate as first author.

Palaiseau, February 6th, 2019

T. Simonson

Thomas Simonson; Professor, Ecole Polytechnique, Palaiseau, France