Identification of the nucleophilic factors and the productive complex for the editing reaction by leucyl-tRNA synthetase

Supplementary data

Identification of the nucleophilic factors and the productive complex for the editing reaction by leucyl-tRNA synthetase

Yohsuke Hagiwara[1,2], Osamu Nureki[3], and Masaru Tateno[1,2]∗

[1]Graduate School of Pure and Applied Sciences, University of Tsukuba, Tennodai 1-1-1, Tsukuba, Ibaraki 305-8571, Japan.

[2]Center for Computational Sciences, University of Tsukuba, Tennodai 1-1-1, Tsukuba, Ibaraki 305-8571, Japan.

[3]Institute of Medical Science, University of Tokyo, Shirokanedai 4-6-1, Minatoku, Tokyo 108-8639, Japan.

Contents

S1. Accessibility of the nucleophile to the C atom

To investigate the dependence of the accessibility of the nucleophile upon the dihedral angle, C2'-C3'-O3'-3'HO, we performed MD simulations with and without rotations of the dihedral angle, and analyzed the O_w−C and O_w−3'-HO distances and the dihedral angle (Fig. S1).

First, as discussed in the text, the trajectory of the 1 ns MD simulation in the absence of any constraints indicates that the average O_w–C distance, 3.4 Å, is still too long for a nucleophilic attack (Fig. S1a), because further approaches of water are prevented by the 3'-HO, which forms a hydrogen bond with the nucleophilic water molecule (see text; Fig. 2b).

Second, in the opened state of the H-gate, the O_w−C distance becomes ~2.4 Å during the MD simulations (Fig. S1b), whereas in the closed state, the distance cannot be shorter than ~2.9 Å (Fig. S1c). These observations are also demonstrated by the comparison of distribution function g(r) of the O_w (see text; Fig. 3).

With respect to the mutant, T248V, the O_w−C distance also becomes ~2.4 Å during the MD simulation for the opened state of the H-gate (Fig. S1d).
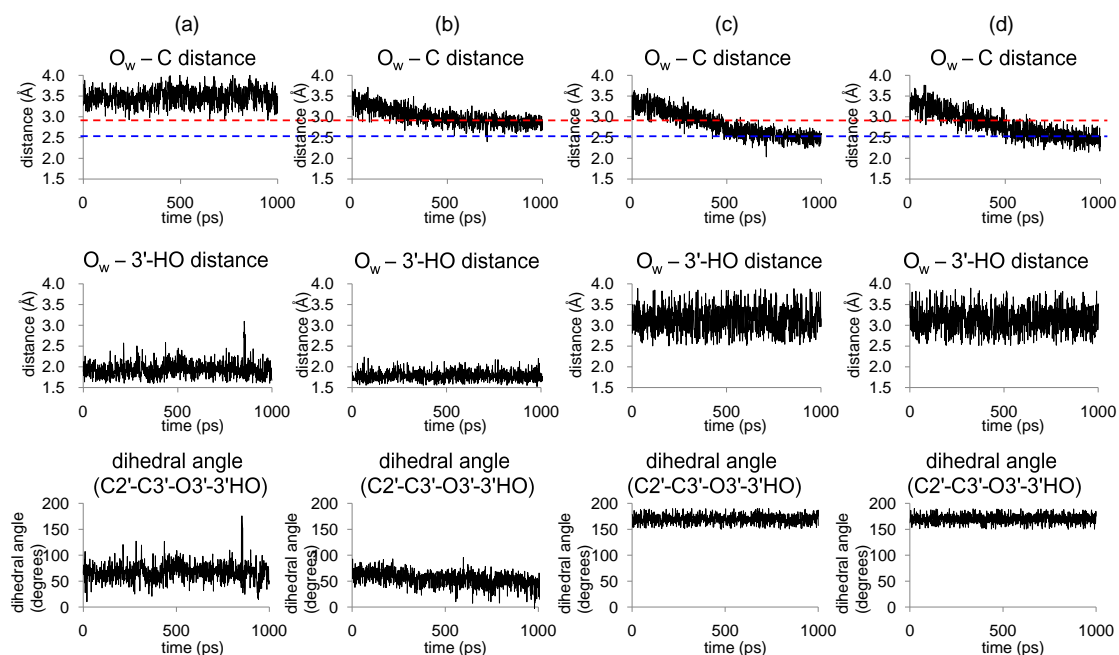
Figure S1. Trajectories of the distance between the O_w and C atoms (Å), the distance between the O_w and 3'-HO atoms (Å) and the dihedral angle C2'-C3'-O3'-3'HO. The trajectories obtained in the 1 ns MD simulation in the absence of any constraints (i.e., the free dynamics) are shown in (a). The trajectories obtained in the constrained MD simulation in the absence and presence of dihedral angle rotation are shown in (b) and (c), respectively. The trajectories obtained in the MD simulation for the T248V mutant, performed in the presence of the dihedral angle rotation, are shown in (d). The red and blue dashed lines denote averaged values of the O_w-C distance calculated using the later 500 ps trajectories.

S2. QM/MM hybrid calculations

2. 1 Computational details

Initial structures for QM/MM calculations were obtained from the optimised structures at the MM level for the two snapshots, corresponding to the closed / opened states (States 1 and 3, respectively) that are mentioned later. All QM/MM calculations were performed using AMBER, GAMESS, and an interface program connecting the two software packages [1-3]. Restricted Hartree-Fock (RHF) / density functional theory (DFT) hybrid all-electron calculations were performed using the B3LYP functional along with the 6-31G* basis set. The link atom approach was used to satisfy valence requirements where the QM/MM boundary separated covalently bonded atoms. While calculating the electrostatic interactions between the QM and MM atoms lying within 25 Å from the center of mass of the QM region, partial charges of the MM atoms were incorporated into the one-electron integral term of the QM Hamiltonian, whereas the interactions between the QM and the other MM atoms were calculated at the MM level. The partial charges were taken from the AMBER parm99 force field.

For geometry optimization, the atoms assigned as QM atoms are as follows: the substrate, i.e., valine-attached A76, the water molecule corresponding to the nucleophile, and the moieties forming hydrogen bonds with the substrate (Thr247, Thr248, the

backbone atoms of Leu328 and Y332, the side chain of Asp347 and the water molecule forming the hydrogen bond with the carbonyl oxygen of the substrate). Thus, 124 atoms, including capping hydrogen atoms, were set as QM atoms. For further calculations where the QM regions were extended to include 527 QM atoms, the following atoms were assigned as QM atoms: the substrate C75, all other atoms of the amino acid residues that form hydrogen bonds with the substrate, the backbone atoms that form covalent bonds with the amino acid residues (Phe246-Thr252, Tyr327-Asp347), and the solvent water molecules surrounding these regions. SCF calculations were performed for States 1 and 3.

2. 2. Investigation of electronic states upon a nucleophilic attack

We investigated changes in the electronic structures of the two states, i.e., the closed state of the H-gate (State 1) where the O_w–C distance is 3.4 Å, and the opened state, where the O_w–C distance is 2.4 Å (State 3). We performed QM/MM combined calculations coupled with the hybrid Hartree–Fock/density functional theory all-electron calculations, using our interface program connecting the QM and MM engines [1]. The geometries of the two states extracted from the MD trajectory were optimised. The slight difference in the total energies between the two states obtained by the geometry

optimisations using the QM/MM scheme (i.e., the energy of State 3 is higher than that of State 1 by 1.8 kcal/mol) assures the feasibility of State 3 obtained by the MM calculations.

Furthermore, in both states, the lowest unoccupied molecular orbital (LUMO) is localised on the carbonyl group of the substrate (Fig. S2), indicating that the C atom can accept electrons from the nucleophile. In this case, the electronic state of the C atom could be the most favourable state for the nucleophilic attack. Here, it should be noted that the character of the antibonding orbital of the C–O$^{2'}$ bond influences the LUMO; this further suggests that this bond would be weakened by the donation of electrons from the nucleophile, thereby leading to cleavage.

However, with respect to the electronic states of the possible nucleophilic water molecule described earlier, significant changes are observed between States 1 and 3. Here, the energy level of the molecular orbital (MO) where the contribution of p-orbitals of O_w is greatest increases from HOMO−19 (State 1) to HOMO−10 (State 3). This corresponds to a decrease of 18.2 kcal/mol in the energy difference between those MOs and LUMOs (Fig. S2). This means that the nucleophilic attack is facilitated by the opening of the H-gate and the formation of the hydrogen bond between $O^{3'}$ and the water. To evaluate the dependence on the size of the QM region, we performed further

QM/MM calculations (within the SCF scheme) for States 1 and 3, in which the QM regions are extended from 124 atoms to 527 atoms. In these calculations, the LUMOs are again localized on the carbonyl group of the substrate in both states, including the antibonding orbital of the C–O2' bond. Moreover, the activation of the nucleophile upon H-gate opening is observed, which agrees well with the results obtained in the calculations including 124 QM atoms.
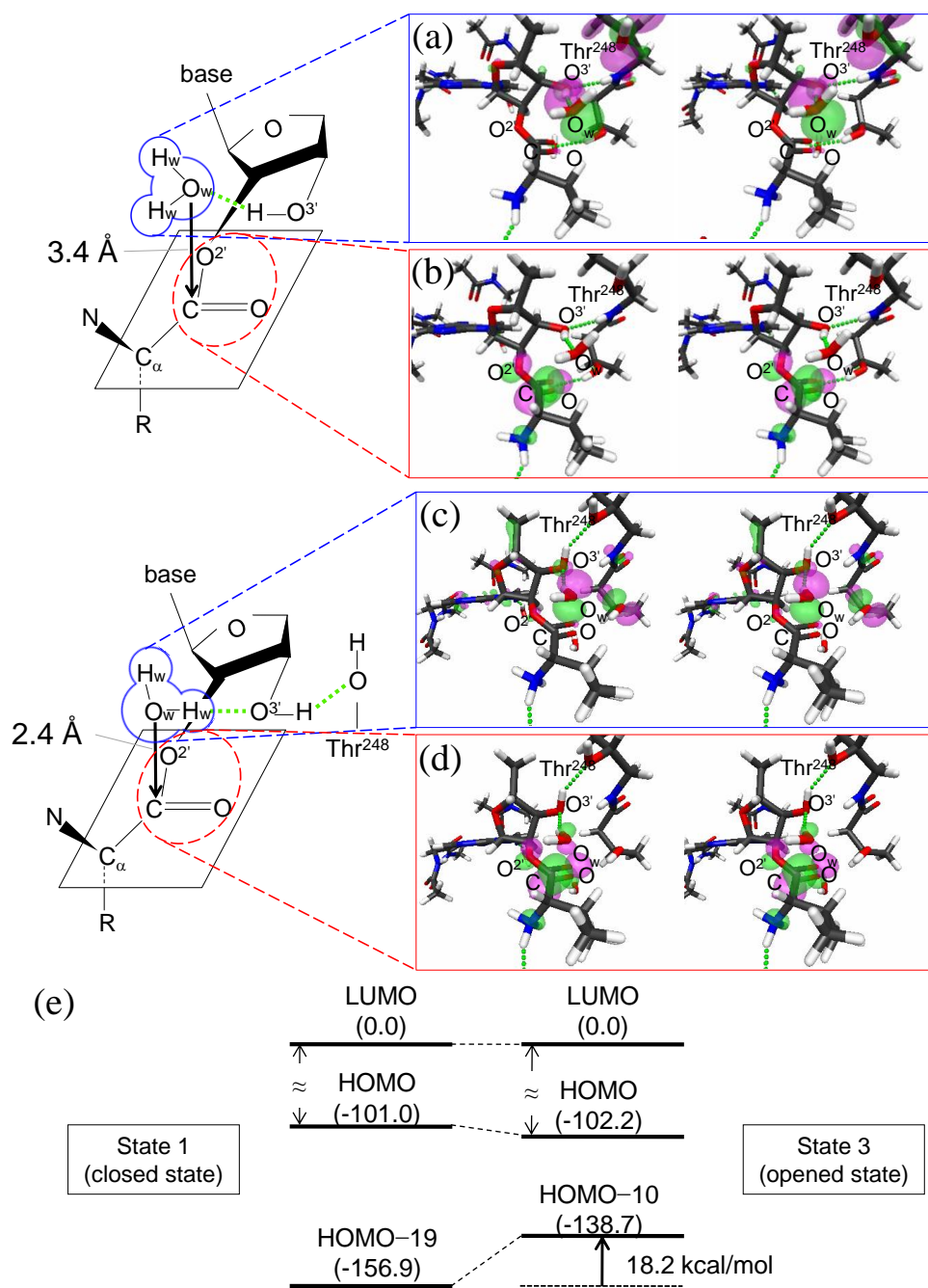
Figure S2. (a), (b) HOMO−19 (a) and LUMO (b) of the structure in which the H-gate is not opened (State 1). (c), (d) HOMO−10 (c) and LUMO (d) of the structure in which the H-gate is opened (State 3). (e) Energy difference between LUMO and HOMO−10 (HOMO−19) in State 1 (State 3) of the H-gate.

S3. Preliminary analysis to estimate the energy barrier in the editing reaction

Our preliminary analysis to obtain the energy barrier for the editing reaction, using QM/MM hybrid simulations, suggested that H-gate opening is likely to be necessary for the reaction to proceed reasonably. In fact, our QM/MM hybrid simulations, which started from distinct states where the H-gate is opened/closed, led to different conclusions concerning the reaction mechanisms: State 3 (the H-gate is opened) leads to reasonable catalytic mechanisms, whereas State 1 (the H-gate is closed) actually leads to a different reaction pathway, which is inconsistent with chemical intuitions. This suggests that the present modelling provides crucial information for further analyses to identify the detailed catalytic mechanisms/pathways of the editing reaction.

S4. References

[1] Schmidt,M.W., Baldridge,K.K., Boatz,J.A., Elbert,S.T., Gordon,M.S., Jensen,J.H., Koseki,S., Matsunaga,N., Nguyen,K.A., Su,S.J., Windus,T.L., Dupuis,M. and Montgomery,J.A. (1993) General atomic and molecular electronic structure system. *J. Comput. Chem.*, **14**, 1347-1363.

[2] Case,D.A., Darden,T.A., Cheatham,T.E. III., Simmerling,C.L., Wang,J., Duke,R.E.,

Luo,R., Merz,K.M., Pearlman,D.A., Crowley,M. *et al.* (2006), AMBER 9, University of California, San Francisco.

[3] Hagiwara,Y., Ohta,T. and Tateno,M. (2009) QM/MM hybrid calculation of biological macromolecules using a new interface program connecting QM and MM engines *J. Phys.: Condens. Matter*, **21**, 064234.