Usage Report for Fiscal Year 2020

Project Title:

Studies on the aminolysis reaction and sterospecificity of papain for the generation of new peptides.

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1. Background and purpose of the project, relationship of the project with other projects

Chemoenzymatic peptide synthesis is an efficient and clean method to generate polypeptides. However, this enzyme-mediated synthesis is dependent on the reaction rate of the protease biocatalyst, which is essentially determined by the natural substrate specificity of the enzyme. Most proteases display high stereo specificity towards L-amino acids, with limited or no reactivity for the D-stereoisomers. However, the incorporation of D-amino acids into peptides is a promising approach to increase they biostability, by conferring intrinsic resistance to proteolysis. D-aminopeptidase (DAP) is one of the few known proteases capable of recognize and use D-amino acids. Therefore, we used quantum mechanics/molecular mechanics (QM/MM) simulations to study the catalytic mechanism of DAP to understand the origin of its stereospecificity. We are also interested in understanding the interaction and internalization of cell-penetrating peptides into lipid membranes.

2. Specific usage status of the system and calculation method

We are using QMMM calculations with a Gaussian09/Amber interface. The atoms treated QM with Gaussian are described with B3LYP/6-31G*, and the MM atoms are described with the ff14SB force field in Amber. We also use the Adaptively Biased Molecular Dynamics (based on metadynamics) as enhanced sampling technique. Additional calculations of lipid membranes and cell-penetrating peptides are carried out using molecular dynamics with AMBER and enhanced sampling techniques such steered molecular dynamics and adaptably biased molecular dynamics.

3. Result

According to experimental results, the calculated energy landscapes with DAP indicate that both l- and D-substrates underwent concerted reactions, being aminolysis the rate-limiting step. L-substrates displayed higher energy barriers for both acylation and aminolysis reactions compared with the energies calculated for D-substrates. In the acylation reaction the interaction between the amino group of the substrate with Asn155 reduced the energetic barrier for D-Ala-OEt, whereas in the aminolysis the orientation of the amino group of L-Ala-OEt towards Asp479 and Asn155 stabilized the acyl-intermediate, increasing the energetic barrier of the reaction.

4. Conclusion

The results of the simulations for DAP and papain are consistent with the experimental evidence, providing a platform for synthesizing polypeptides that incorporate D-amino acids and help clarify the stereospecificity of proteases.

5. Schedule and prospect for the future

Studies on cell-penetrating peptides with d-amino acids or non-proteonogenic amino acids are ongoing to study their stability and penetration abilities. We would like to extend the use of the supercomputer of the Quick User account to fiscal year 2021

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Fiscal Year 2020 List of Publications Resulting from the Use of the supercomputer

[Paper accepted by a journal]

Joan Gimenez-Dejoz J, Kousuke Tsuchiya, Ayaka Tateishi, Yoko Motoda, Takanori Kigawa, Yasuhisa Asano, Keiji Numata. Computational study on the polymerization reaction of D-aminopeptidase for the synthesis of D-peptides. RSC Advances. **10**, 17582-17592.

Kayo Terada, Joan Gimenez-Dejoz, Taichi Kurita, Kazusato Oikawa, Hirotaka Uji, Kousuke Tsuchiya*, Keiji Numata*. Synthetic mitochondria-targeting peptides incorporating α-aminoisobutyric acid with a stable amphiphilic helix conformation in plant cells. *ACS Biomaterials Science and Engineering*, **accepted**.

[Oral presentation]

- Insights into the Stereospecificity of proteases for L- and D-amino acids in Chemoenzymatic Polymerization from Quantum Mechanics/Molecular Mechanics Simulations. Joan Gimenez-Dejoz, Ayaka Tateishi, Yoko Motoda, Kousuke Tsuchiya, Keiji Numata. 69th SPSJ Symposium on Macromolecules.
- 移行効率向上を指向したα-アミノイソブタン酸含有ミトコンドリア移行配列の創製. 寺田 佳世、Gimenez Joan 、土屋 康佑、沼田 圭司. 第 16 回京大植物縦横無尽の会、web 開催、2020 年 11 月 13 日.