Usage Report for Fiscal Year 2017

Project Title:

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

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Description of the project

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

Chemoenzymatic synthesis of polypeptides has attracted recently great deal of interest for developing new biomedical applications and pharmaceutical materials. It consists in the use of proteases to synthesize new polypeptides and has become an alternative to the chemical synthesis. However, many molecular aspects of the polymerization reaction and the key factors that control the selectivity of the proteases are still unknown. This lack of knowledge is hampering the advance of the field, limited by the natural specificity and efficiency of the enzymes. To achieve the creation of polypeptides with novel functionalities, new genetic engineered proteases, with modified catalytic activity allowing both the incorporation of unnatural amino acids and increasing its efficiency, are required. To accomplish this objective, we are using a combined approach of well-established laboratory experiments and analytical methods together with theoretical studies of the polymerization reaction, consisting of two steps, acylation and aminolysis, of the model cysteine protease papain with the substrates L- and D-alanine ethyl ester, to understand the remarkable different specificity of the enzyme for these substrates.

2. Specific usage status of the system and calculation method

We are using the supercomputer to model the polymerization reaction using QM/MM with Adaptatively Biased Molecular Dynamics with Gaussian and AMBER.

3. Result

We are performing QM/MM MD simulations for both steps of the reaction and for both substrates. Partial results indicate that the free energy barrier for the acylation step is similar for both L- and D-alanine ethyl ester. Preliminary QM results seem to indicate that the aminolysis reaction with L-alanine ethyl ester presents lower free energy barriers when compared with D-alanine ethyl ester.

4. Conclusion

The crucial difference between the reactions with L- and D-alanine ethyl ester occurs in the aminolysis step, in agreement with experimental results.

5. Schedule and prospect for the future

We plan to finish the calculations for this enzyme and substrates and publish the results. We also want to simulate the aminolysis reaction with different intermediates and substrates to examine the determinants of the stereospecificity of papain. Additionally, we plan to model the enzyme D-aminopeptidase to examine its unique specificity toward D-amino acids. I have a Quick user account and I would like to continue using the system for the fiscal year 2018 to complete the simulations.