從分子角度探討經 SpyTag/SpyCatcher 環化對於不同酵素之穩定性影響

黃乙淇 (5121)

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大綱

摘要

酵素在高溫下穩定性差、容易失活等缺點，為了使酵素能廣泛應用於大規模工業發展，熱穩定性是首要改善的目標之一，蛋白質環化已成為提高酵素熱穩定性的方法。本次報告主要探討透過 SpyTag/SpyCatcher 環化是否能改善酵素在使用上的缺點，並從分子層面如分子結構、分子動力學模擬等角度解釋環化影響酵素熱穩定性的原因。根據文獻結果指出使用 SpyTag/SpyCatcher 能成功環化並表現 β-葡萄糖苷酶、海藻糖合成酶 (TreS) 及地衣多醣酶，其熱穩定性相較游離酵素皆有提升。在 β-葡萄糖苷酶部分也針對胜肽標籤的融合順序是否對環化造成影響進行實驗，透過分子結構模擬分析可知優先轉譯 SpyTag 能幫助蛋白質折疊並環化成良好構型；在海藻糖合成酶部分，額外使用定點突變的方式與環化酵素比較 pH 值、溫度、酵素動力學及熱穩定性差異，並透過分子動力學模擬解釋熱穩定性增加的原理。為了觀察地衣多醣酶和 SpyTag/SpyCatcher 的界面間的相互作用，以突變方式改變胺基酸帶電型態，根據結果可知，靜電力可能在蛋白質辨識過程中發揮靜電引導作用，可促進蛋白質-蛋白質相互作用的形成並提供方向性，對於熱穩定性會有更大的影響。綜合三篇文獻結論，從分子層面能更進一步了解環化酵素影響熱穩定性的原因，更能合理的解釋在熱穩定性數據上的差異。

Exploring the impact of SpyTag/SpyCatcher cyclization on the stability of different enzymes from a molecular perspective

Yi-Chi Huang (5121)

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Outline

1. Introduction
2. Rational design of spontaneous self-cyclization enzymes *In Vivo* and *In Vitro* with improved thermal tolerance and activity
3. Improving the thermostability of trehalose synthase from *Thermomonospora curvata* by covalent cyclization using peptide tags and investigation of the underlying molecular mechanism
4. Protein-protein interactions enhance the thermal resilience of SpyRing-cyclized enzymes: A molecular dynamic simulation study
5. Conclusion

Abstract

Enzymes typically exhibit poor stability at high temperatures and tend to lose activity, which is a significant drawback limiting their broad application in large-scale industrial processes. Improving thermal stability is the primary objectives to overcome this limitation. Protein cyclization has emerged as an effective method to enhance enzyme thermal stability. This report examines whether SpyTag/SpyCatcher cyclization can improve enzyme limitations, focusing on molecular mechanisms that influence thermal stability, such as structural changes and molecular dynamics. Literatures show successful application of this method to enzymes like β-glucosidase, trehalose synthase, and lichenase, all of which exhibited enhanced thermal stability compared to non-cyclized versions. For β-glucosidase, experiments were conducted to investigate whether the order of peptide tag fusion affects the cyclization process. Molecular structure simulations showed that translating SpyTag first helps with protein folding and leads to a more stable conformation. For trehalose synthase, additional experiments were performed comparing enzymes with site-directed mutations to their cyclized versions, focusing on differences in pH, temperature, enzyme kinetics, and thermal stability. Molecular dynamics simulations were used to explain the underlying mechanism of enhanced thermal stability. To examine the interactions between lichenase and the SpyTag/SpyCatcher interface, mutations were introduced to alter the charge properties of amino acids. The results indicated that electrostatic forces might play an electrostatic steering role during protein recognition, promoting protein-protein interactions and providing directionality, which has a more significant effect on thermal stability. In conclusion, these three studies provide insights into the molecular mechanisms by which enzyme cyclization influences thermal stability, offering a reasonable explanation for the observed differences in thermal stability data.

參考文獻

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