利用飽和定點突變對氧化鯊烯環化酵素內假設活性區域殘基進行結構—反應關係之研究

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摘要

氧化鯊烯環化酵素(oxidosqualene-lanosterol cyclase, ERG7)在動物、真菌及高等植物體內催化非環狀的氧化鯊烯 (oxidosqualene)環化成為四環或五環的三萜帖類(Triterpnoids)。由氧化鯊烯環化酵素所一步催化而產生的一連串環化/重排反應，在這過半個世紀以來都一直令有機生物學家著迷。在本研究課題中，我們已經成功將啤酒酵母菌(*Saccharomyces cerevisiae*)中的Tyr510 和Phe699 等位置適合在羊毛脂醇生成中扮演穩定碳陽離子的轉移和成環反應之芳香族胺基酸，做飽和定點突變並進行反應與結構關係相關研究。在ERG7^{Y510X}的飽和定點突變株內產生包含achilleol A、camelliol C、 (13*αH*)-isomalabarica-14(26)*E*,17,21-trien-3β-ol、lanosterol和parkeol等產物。這些結果暗示Tyr510 可能扮演了穩定在環化/重排反應串中所產生的單環C-10 碳陽離子、反馬可尼可夫C-14 碳陽離子(anti-Markevoikov C-14 cation)以及羊毛脂醇碳陽離子中間物（lanosteryl cation）的重要角色。

另外在ERG7^{F699X}的飽和定點突變株實驗中，除了Thr、Met、Pro、Leu、Ile、His等胺基酸取代基外，絕大部分的取代基都喪失了完整的環化酵素功能，這結果暗示該殘基位置對氧化鯊烯環化酵素的重要性。近一步地，我們首次在ERG7 突變株發現三種新產物包含 prososta-13(17),24-dien-3β-ol 、prososta-17(20),24-dien-3β-ol和malabarica-14*E*,17,21-trien-3β-ol等。除此之外還包括(13*αH*)-isomalabarica-14*Z*,17,21-trien-3β-ol 與

(13*αH*)-isomalabarica-14*E*,17,21-trien-3β-ol這些在椅式—船式(C-B)或椅式—椅式6-6-5 三環碳陽離子或羊毛脂醇碳陽離子環化重排時提前終止所產生的中間產物。整合以上結果證明了Phe699 在氧化鯊烯環化酵素內不僅扮演了穩定反馬可尼可夫C-14 碳陽離子以及羊毛脂醇C-17 碳陽離子中間物外更擁有改變氧化鯊烯環化酵素內活性區結構及其之後的反應的可塑性。

Studies of the Structure-Reactivity Relationships on the Putative Active Site Cavity Residues of Oxidosqualene-Lanosterol Cyclase by Site-Saturated Mutagenesis

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Abstract

Oxidosqualene-lanosterol cyclases (ERG7) catalyze the conversion of acyclic oxidoaqualene into tetracyclic or pentacyclic triterpenoids in the animals, fungi, and high plants. A serial cyclization/rearrangement cascades catalyzed in one-step reaction by oxidosqualene-lanosterol cyclase have fascinated the bioorganic researcher over a half century. We have successfully performed the site-saturated mutagenesis on the Tyr510 and Phe699 residues of *Saccharomyces cerevisiae* ERG7, corresponding to the appropriate orientations to stabilize the carbocation intermediates in the lanosterol formation. The site-saturated ERG7^{Y510X} mutants produced achilleol A, camelliol C, (13α*H*)-isomalabarica-14(26)*E*,17,21-trien-3β-ol, lanosterol and parkeol. The results suggested that the position of Tyr510 residue may play a crucial role in stabilizing the monocyclic C-10 cation anti-Markovnikov C-14 cation intermediate and lanosteryl cation that were generated in the cyclization/rearrangement cascades.

In the cases of the site-saturated ERG7^{F699X} mutants, most of the substituted residues failed to complement the cyclase activity, except the Thr, Met, Lys, Asn, and His, indicating the importance of the position for the catalytic function of ERG7. Further, three novel intermediates, protosta-13(17),24-dien-3β-ol, protosta-17(20),24-dien-3β-ol, and malabarica-14*E*,17*E*,21-trien-3β-ol, were isolated, for the first time, from the

ERG7^{F699M} mutant. These products, in addition to (13α*H*)-isomalabarica-14*Z*,17*E*,21-trien-3β-ol and (13α*H*)-isomalabarica-14*E*,17*E*,21-trien-3β-ol, corresponding to truncation of the cyclization/rearrangement cascade at the chair-boat (C-B) or chair-chair (C-C) 6-6-5 tricyclic cation and/or lanosteryl C-17 cation. Taken together, these results demonstrate the functional role of the ERG7 Phe699 residue in stabilizing Markovnikov tricyclic C-14 and lanosteryl C-17 cations as well as the plasticity of the ERG7 mutant enzyme in changing the active site structure and subsequent reaction cascade.

Abbreviations

AACT	acetoacetyl-CoA thiolase
CAS	cycloartenol synthase
DMAPP	dimethylally diphosphate
FPS	farnesyl diphosphate synthase
GPP	geranyl diphosphate
GPS	geranyl diphosphate synthase
HMGS	3-hydroxy-3-methylglutaryl-CoA synthase
HMGR	3-hydroxy-3-methylglutaryl-CoA reductase
IPI	isopentenyl diphosphate isomerase
IPP	isopentenyl diphosphate
LAS	lanosterol synthase
MK	mevalonate kinase
MVD	mevalonate diphosphate decarboxyase
OS	oxidosqualene
OSC	oxidosqualene cyclase
PMK	phosphomevalonate kinase
SHC	squalene-hopene cyclase
SQE	squalene epoxidase
SQS	squalene synthase
TLC	Thin-layer-chromatgraphy

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