Trichoderma koningii G-39中雙功能阿拉伯呋喃糖苷酵素/木糖苷酵素的過量表現、突變以及反應機制的研究

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摘 要

本研究旨在探討源自於木腐黴菌 *Trichoderma koningii* G-39（也稱 *Hypocrea koningii* G-39）之阿拉伯呋喃糖苷酵素的性質、催化功能、反應機制與重要胺基酸的鑑定。

Trichoderma koningii G-39 之Abf屬於醣苷水解酵素第 54 家族，本研究首次成功地將該基因表現於*Pichia pastoris*酵母菌系統。以SP陽離子交換層析管柱可純化得均質度＞90%之重組酵素，研究顯示該重組酵素為雙功能酵素，同時具有阿拉伯呋喃糖苷酵素(Abf, EC 3.2.1.55)及木糖苷酵素(xyl, EC 3.2.1.37)之活性。利用抑制研究初步推測阿拉伯呋喃糖苷酵素及木糖苷酵素反應作用於不同的活化位置。我們也進一步探討其催化反應機制，由NMR光譜及動力學分析的研究結果，顯示Abf酵素是屬於保留構型之催化機制，當Abf與不同受質作用時，其催化是經由arabinosyl-enzyme中間體的形成，以兩步驟雙取代反應之機制進行。由胺基酸序列比對以及

同一家族的Abf（*Aspergillus kawachii*）三度空間結構之比對，我們將其中具高度保留的 24 個殘基進行定點突變及動力學的研究，結果指出*T. koningii* G-39 Abf酵素催化反應的親核性基團及一般酸鹼催化基團分別為E223 及D299 殘基，而D221 殘基則具有穩定受質鍵結之功能。以原型酵素（wild-type）與D299G突變株進行一系列不同受質的活性分析，測得其Brønsted constant (β_{lg})分別為– 0.18 和–1.3，顯示在原型酵素之催化反應中，去阿拉伯糖基化(dearabinosylation step)為速率決定步驟，然而當D299殘基被取代後，反應之速率決定步驟即變為酵素糖基化之步驟，此等結果顯示D299 殘基在Abf催化反應中扮演一般酸/鹼（general acid/ base）之角色，此論點亦可由D299N之pH-profile結果得到印證。

Overexpression, mutagenesis and mechanistic study of a bifunctional α-L-arabinofuranosidase/β-D-xylosidase from *Trichoderma koningii* G-39

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Abstract

A gene from *Trichoderma koningii* G-39 encoding an enzyme with α-L-arabinofuranosidase/β-D-xylosidase (Abf; member of the GH54 family) activity was expressed in *Pichia pastoris*. The recombinant wild-type enzyme and mutants were purified to > 90% homogeneity by cation-exchange chromatography. Extensive mutagenesis of 24 conserved Glu and Asp residues of family 54 was performed. The k_{cat} values of the D221N and D299N were 7000- and 1300-fold lower than that of the wild-type Abf, respectively, while E223Q was completely inactive. These results are consistent with implications from the *Aspergillus kawachii* α-L-arabinofuranosidase three-dimensional structure. This structure indicates that E223 of *T. koningii* Abf function as a nucleophile and D299 as a general acid/base catalyst for the enzymatic reaction. D221 in Abf is significant for substrate binding. The catalytic mechanism of wild-type Abf was further investigated by NMR spectroscopy and kinetic analysis. The results showed that Abf is a retaining enzyme. It catalyzes various substrates via the formation of a common intermediate that is probably an

arabinosyl-enzyme intermediate. A two-step, double-displacement mechanism involving first the formation, and then the breakdown, of an arabinosyl-enzyme intermediate was proposed. Based on the k_{cat} values of a series of aryl-α-L-arabinofuranosides catalyzed by wild-type Abf, a relatively small Brønsted constant, $\beta_{lg} = -0.18$, was obtained, suggesting that the rate-limiting step of the enzymatic reactions for substrates (where the leaving phenols have pK_a values ≥ 5.15) is the dearabinosylation step. Further kinetic studies with D299G mutant revealed that the catalytic activity of this mutant depended largely on the pK_a values (> 6) of leaving phenols, with $\beta_{lg} = -1.3$. This indicated that the rate-limiting step became arabinosylation step when D299G was employed. This kinetic outcome supports the idea that D299 is the general acid/base residue. The pH activity profile of D299N provided further evidence strengthening this suggestion.