家族 54 阿拉伯呋喃糖苷酵素之反應機制探討

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摘　　要

本研究利用之前已發展完善的純化流程，純化出 α-*L*-阿拉伯呋喃糖苷酵素，均質度在 90% 以上，酵素單體分子量約 50 kDa。

針對 α-*L*-阿拉伯呋喃糖苷酵素，我們合成一系列含有不同離去基之受質以動力學之研究以探討其特異性及反應機制。同時也合成此酵素之可能抑制劑，1,2-epoxy-3-(α-*L*-arabinofuranosyl)propane 進行抑制反應研究。

以不同受質與酵素進行催化反應，在反應系統內加入相同比例之甲醇讓酵素同時進行轉醣反應，反應結果顯示酵素以構型保留之方式進行催化，由 NMR 光譜分析得知酵素催化的過程經過共同的反應中間體 (common intermediate)，此中間體可能是阿拉伯醣基化酵素中間體。

以受質離去基之 pK_a 與 log k_{cat} 及 log k_{cat} / K_m 作圖可得 Brønsted plot。由 Brønsted plot 及共同反應中間體可推論此酵素之催化機構為兩步驟取代反應，即為酵素醣基化(arabinosylation)及去醣基化(dearabinosylation)。由於其 β_{lg}=-0.19 左右，顯示離去基的強弱在催化過程中並非速率決定步驟，以蛋白質結構分析得知 E223 與 D299 是此酵素重要胺基酸殘基，我們因此以 D299G 突變酵素進行 Brønsted plot 研究，並得 β_{lg}=-1.3 左右，顯示除去 D299 後催化反應之速率決定步驟改變為醣基化步驟。

很可惜的是 1,2-epoxy-3-(α-*L*-arabinofuranosyl)propane 無法與酵素作親和性的標誌，無法明確地用以定位重要胺基酸，但其為競爭形抑制劑， K_i 值為 4.85 mM。

Mechanistic study of family 54 α-*L*-arabinofuranosidase

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ABSTRACT

The recombinant enzyme, α-*L*-arabinofuranosidase (ABF), was efficiently purified by a well developed method to more than 90% homogeneity by SP (cation) column. The molecular weight of the enzyme is about 50 kDa by SDS page.

In order to investigate the catalytic mechanism of the ABF enzyme a series of substrate have been synthesized. In addition, a compound containing epoxide ring was synthesized for an irreverseible inhibitor to ABF enzyme.

Hydrolyses of different substrates within the same ratio of methanol and water are catalized by ABF enzyme. Depending on NMR spectra, the result shows that in different substrates catalytic procedures all pass through a common intermediate. By transglycosylation of ABF enzyme was shown to cleave the glycosidic bond with a retention of the anomeric configuration.

Measurement of V_{max} and K_m values for a series of aryl-α-*L* -arabinofuranoside allowed constructing a Brønsted plot. Brønsted plot and the common intermediate indicate that the enzyme catalyzes the reaction with a two-step mechanism involving the formation and breakdown of arabionfuransyl-enzyme intermediate. By Brønsted plot efficiency β_{lg}=-0.19 we know that the ability of leaving group is not the rate determine step thus the rate determine step is dearabinofuranosylation step. We indicate that E223 and D299 are the essential residues of α-*L*-arabinofuranosidase by protein structure. We also constructing a mutant enzyme D299G Brønsted plot. Its efficiency β_{lg} is -1.3. It means that the rate determine step of enzyme changes to arabinofuranosylation without D299.

Unfortunately the inhibitor of ABF enzyme can not be labeled on the essential amino acids. Futher, investigation of the relationship between enzyme and inhibitor by double reciprocal plot, indicated that the inhibitor is a competitive inhibitor.