

เอนไซม์และยีนของเจอร์รานิลเจอร์รานิล ไดฟอสเฟต ฟอสฟาเตส
ในวิถีชีวสังเคราะห์ของเปลาโนทอลในเปล้าน้อย



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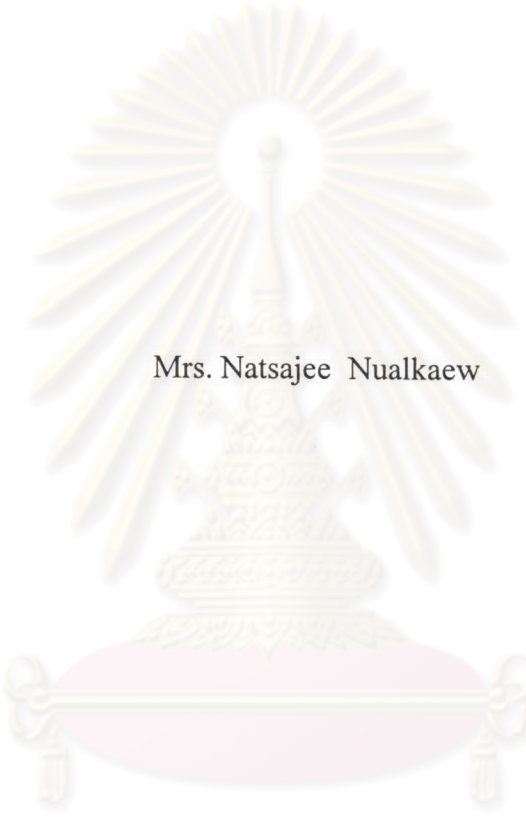
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GERANYLGERANYL DIPHOSPHATE PHOSPHATASE ENZYME AND
GENE OF PLAUNOTOL BIOSYNTHETIC PATHWAY
IN *CROTON STELLATOPILOSUS* OHBA



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นาฏคจี นวลแก้ว : เอนไซม์และยีนของเจอร์รานิลเจอร์รานิลไดฟอสเฟต ฟอสฟาเตส ในวิถีชีวสังเคราะห์ของเปลลาโนทอลในเปล้าน้อย (GERANYLGERANYL DIPHOSPHATE PHOSPHATASE ENZYME AND GENE OF PLAUNOTOL BIOSYNTHETIC PATHWAY IN *CROTON STELLATOPILOSUS* OHBA) อาจารย์ที่ปรึกษา : รศ.ดร. วันชัย ดีเอกนามกุล, อาจารย์ที่ปรึกษาร่วม : Professor Dr. Meinhart H. Zenk 147 หน้า. ISBN 974-53-2091-9.

การป้อนสารติดฉลากของเจอร์รานิลเจอร์รานิล [1-³H]GGOH เข้าสู่ใบและก้อนคลัสต์ของเปล้าน้อย พบว่าสารติดฉลากถูกนำไปใช้ในการสร้างเปลลาโนทอล จากการศึกษาด้านเอนไซม์ทำให้สามารถตรวจพบกิจกรรมของ เอนไซม์เจอร์รานิลเจอร์รานิลไดฟอสเฟต ฟอสฟาเตส (GGDP phosphatase) ได้ทั้งในสารสกัดเอนไซม์จากใบและในคลอโรพลาสต์ เมื่อทำการแยกเอนไซม์เบื้องต้น โดยวิธีเจลฟิลเดชัน โครมาโตกราฟี ทำให้พบว่า GGDP phosphatase ในสารสกัดเอนไซม์ มีอยู่สองชนิดคือ PI และ PII การศึกษาคุณสมบัติของเอนไซม์ทำให้พบว่า PI มีน้ำหนักโมเลกุล 232 kD ประกอบด้วย 4 หน่วยย่อยของโปรตีนที่มีขนาด 58 kD โดยมีค่า Km 0.2 mM ค่า Vmax 277.8 pkat/mg protein และมีสภาวะการทำงานเหมาะสมที่ pH 6.0-6.5 ในขณะที่เอนไซม์ PII เป็นโปรตีนเดี่ยวที่มีน้ำหนักโมเลกุล 30-34 kD โดยมีค่า Km 0.1 mM ค่า Vmax 7.5 nkat/mg มีสภาวะการทำงานเหมาะสมที่ pH 6.5-7.0 และถูกยับยั้งการเร่งปฏิกิริยาได้โดยสารตั้งต้นที่ความเข้มข้นสูง นอกจากนี้ยังพบว่า PI และ PII เป็นเอนไซม์ที่เกาะติดกับผนังเซลล์ โดยกิจกรรมเอนไซม์สามารถถูกยับยั้งได้โดยโมลิบเดตอออน เอนไซม์ทั้งสองมีความจำเพาะของการใช้เจอร์รานิลเจอร์รานิลไดฟอสเฟตสูงที่สุด เมื่อเปรียบเทียบกับการใช้สารตั้งต้น เจอร์รานิลฟอสเฟต และฟานสซิลไดฟอสเฟต การศึกษากระบวนการเร่งปฏิกิริยาของเอนไซม์พบว่า การตัดหมู่ไดฟอสเฟตออกจากเจอร์รานิลเจอร์รานิลไดฟอสเฟต โดยเอนไซม์ PI และ PII เกิดขึ้นโดยผ่านปฏิกิริยา 2 ขั้นตอนคือจากสารตั้งต้นเจอร์รานิลเจอร์รานิลไดฟอสเฟต เปลี่ยนไปเป็นเจอร์รานิลเจอร์รานิลโมโนฟอสเฟต และเจอร์รานิลเจอร์รานิลออล ตามลำดับ การโคลนยีนจากใบเปล้าน้อย โดยอาศัยข้อมูลของลำดับกรดอะมิโนของพรีนัลไดฟอสเฟต ฟอสฟาเตส จากฐานข้อมูล Swiss-Prot ทำให้ได้ยีนที่สามารถถอดรหัสโปรตีนขนาด 33.6 kD และยังคงแสดงกิจกรรมเอนไซม์เจอร์รานิลเจอร์รานิลไดฟอสเฟต ฟอสฟาเตส เอนไซม์ฟอสฟาเตสที่แสดงออกนี้ มีกระบวนการเร่งปฏิกิริยาการเปลี่ยนจาก GGDP ไปเป็น GGOH ในแบบ 2 ขั้นตอนเช่นเดียวกับเอนไซม์ GGDP phosphatase ที่แยกได้จากส่วนใบ คือจาก GGDP ผ่าน GGMP ไปเป็น GGOH ในที่สุด

สาขาวิชา เกษษเคมีและผลิตภัณฑ์ธรรมชาติ
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ลายมือชื่อนิสิต.....
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KEY WORD: *CROTON STELLATOPILOSUS* / GERANYLGERANIOL / GERANYLGERANYL
DIPHOSPHATE / GERANYLGERANYL DIPHOSPHATE PHOSPHATASE / PLAUNOTOL
BIOSYNTHESIS / PRENYL DIPHOSPHATE PHOSPHATASE

NATSAJEE NUALKAEW : GERANYLGERANYL DIPHOSPHATE PHOSPHATASE
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The whole leaves and callus cultures of *Croton stellatopilosus* Ohba could incorporate [$1\text{-}^3\text{H}$]geranylgeraniol into plaunotol. Enzymological studies showed that geranylgeranyl diphosphate phosphatase (GGDP phosphatase) activity was present in cell-free extracts of the leaves and of chloroplasts. The crude enzyme extract could be separated into two peaks of GGDP phosphatase activities (PI and PII) by gel filtration. Purification and characterization of both enzyme peaks revealed that PI was a tetrameric enzyme of 232 kD with its subunit size of 58 kD. It showed optimum pH of 6.0-6.5, an apparent K_m of 0.2 mM, and a V_{max} of 277.8 pkat/mg. PII was a monomeric enzyme of 30-34 kD with an apparent K_m value of 0.1 mM, V_{max} of 7.5 nkat/mg and exhibited substrate inhibition. Both PI and PII appeared to be membrane-bound enzymes and their activities could be inhibited by Mo^{2+} . Both preferred GGDP as their substrate rather than geranyl diphosphate or farnesyl diphosphate. Study on the catalysis of the reaction revealed that GGOH was formed from GGDP by two successive monodephosphorylations, rather than a one-step diphosphate dephosphorylation. Cloning of the phosphatase genes from *C. stellatopilosus* leaves, based on the available information of prenyl diphosphate phosphatase in Swiss-Prot database, was also performed using *E. coli* as expression system. The results showed that the encoded protein had its molecular weight of 33.6 kD and could exhibit GGDP phosphatase activity. The expressed phosphatase also showed its process of catalytic dephosphorylation by using two sequential steps of monophosphate dephosphorylation from GGDP to GGMP and to GGOH.

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ABBREVIATIONS

%	=	percent (part per 100); percentage
β	=	beta
β -	=	beta-
δ	=	chemical shift
μCi	=	microCurie
μl	=	microliter (s)
μM	=	micro molar (s)
λ_{max}	=	wavelength at maximum absorption
μmol	=	micromole
/	=	per, or divided by
[1- ¹⁴ C]	=	carbon position 1 of the molecule is labeled with carbon 14
[1- ³ H]	=	hydrogen position 1 of the molecule is labeled with tritium
¹ H-NMR	=	proton nuclear magnetic resonance
2,4-D	=	2,4-dichlorophenoxyacetic acid
Bis	=	N,N'-dimethyl bisacrylamide
bp	=	base pair
BSA	=	bovine serum albumin
cDNA	=	cloning DNA
cDNA	=	complementary deoxyribonucleic acid
CHCl ₃	=	chloroform
Ci	=	Curie
cm	=	centimeter
cpm	=	counts per minute
CTP	=	cytidine 5'-triphosphate
Da	=	Dalton, unit of molecular mass (1/12 of C=1)
DNA	=	deoxyribonucleic acid
dpm	=	disintegrations per min
EDTA	=	ethylenediamine tetraacetic acid
e. g.	=	for example
EM	=	electron microscope

etc.	=	et cetera
Fig	=	Figure
g	=	gram
<i>g</i>	=	centrifugal force (relative to gravity)
GGPPS	=	geranylgeranyl pyrophosphate synthase
hr	=	hour
Hz	=	Hertz
IDP	=	isopentenyl diphosphate
kD	=	kilodalton, ($\times 10^3$ Da)
kg	=	kilogram
K_m	=	Michaelis constant = substrate concentration at which the rate of enzyme-catalysed reaction is half maximum rate
l	=	liter (s)
LS	=	Linsmair and Skoog (1965) medium
m	=	meter (s)
M	=	molar (s)
m/z	=	mass to charge ratio
mA	=	milliampere
MeOH	=	Methanol
mg	=	milligram
ml	=	milliliter
mm	=	millimeter
mM	=	millimolar
mmol	=	millimole
<i>Mr</i>	=	relative molecular mass
MS	=	Murishage and Skoog (1962) medium
MS	=	Mass spectrometry
MW	=	molecular weight
N ₂	=	Nitrogen atmosphere
NaOH	=	sodium hydroxide
nkat	=	nano katal

nm	=	nanometer (s)
no.	=	number
°C	=	degree Celsius
opt	=	optimum
PCR	=	polymerase chain reaction
pH	=	hydrogen ion concentration
pI	=	isoelectric point
pkat	=	pico katal
pmol	=	picomole (s)
ppm	=	part per million
q.s.	=	quantum suffices
R _f	=	distance spot moved/ distance solvent moved (TLC)
RNA	=	ribonucleic acid
rpm	=	revolutions per minute
SDS	=	sodium dodecyl sulfate
SDS-PAGE	=	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
sec	=	second (s)
sp.act.	=	specific activity
TEMED	=	N,N,N',N'-tetramethylethylenediamine
TLC	=	thin layer chromatography
UV	=	ultraviolet light
v/v	=	volume/ volume
V _e	=	elution volume
V _{max}	=	maximum velocity of enzyme
V _o	=	void volume
w/v	=	weight/volume (concentration)
wt	=	weight