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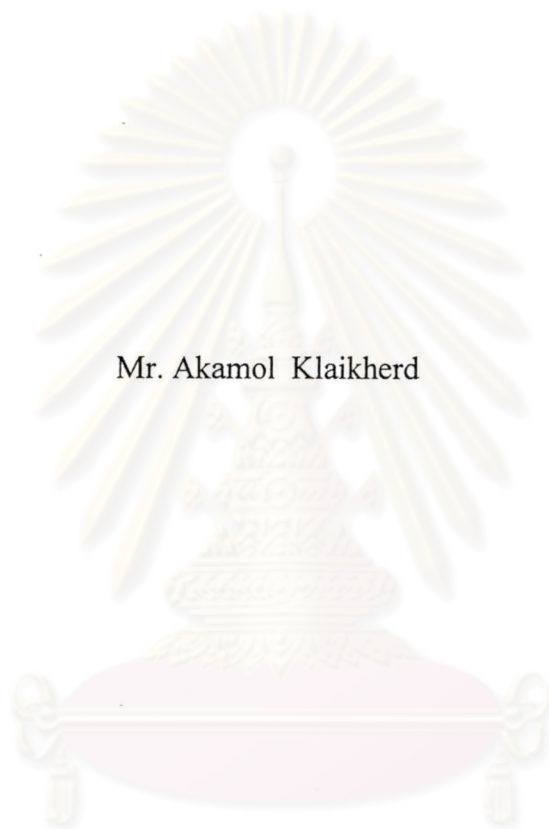
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PREPARATION OF *N*-ACETYL-D-GLUCOSAMINE AND CHITOLIGOSACCHARIDE
BY ENZYMATIC HYDROLYSIS OF CHITIN AND CHITOSAN WITH SERUM
FROM PARA RUBBER



Mr. Akamol Klaikherd

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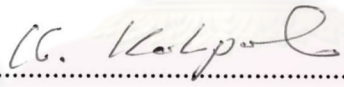
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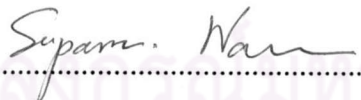
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เอกภมด คล้ายเกิด: การเตรียมเอ็น-แอสีทิล-ดี-กลูโคซามีนและไคโทโอลิโกแซ็กคาไรด์โดย
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C HYDROLYSIS OF CHITIN AND CHITOSAN WITH SERUM PROM PARA
RUBBER) อ. ที่ปรึกษา: ผศ. ดร. มงคล สุขวัฒนาสินิทธิ์; อ.ที่ปรึกษาร่วม: รศ.ดร. ศุภสร
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ซีรัมจากยางพารา (*Hevea Brasiliensis*) เป็นผลพลอยได้จากกระบวนการผลิตน้ำยางข้นซึ่ง
มีเอนไซม์ที่สามารถย่อยไคตินแบบเอ็นโดไคทิเนสมีชื่อว่า เฮวามีน (hevamine) งานวิจัยนี้เสนอ
ความเป็นไปได้ในการนำเอนไซม์จากซีรัมยางพารามาใช้ผลิตน้ำตาลเอ็น-แอสีทิล-ดี-กลูโคซามีน
(GlcNAc) และน้ำตาลเอ็น,เอ็น-ไดแอสีทิลไคโทไบโอส [(GlcNAc)₂] จากบีตาไคติน โดยซีรัม
ยางพาราที่ใช้ในการทดลองนี้มีปริมาณ โปรตีนทั้งหมด 6 mg/mL และมีแอกทิวิตีการย่อยไคตินอยู่ที่
18 มิลลิวินิต (mU) ต่อมิลลิกรัม โปรตีน อัตราส่วนที่เหมาะสมระหว่างซีรัมต่อไคตินเป็น 0.22 มิลลิ
วินิต/มิลลิกรัม โดยความเข้มข้นของไคตินที่เหมาะสม คือ 60 มิลลิกรัม/มิลลิลิตร ช่วงความเป็นกรด
ด่างที่เหมาะสมของสารละลายคือ pH 2-4 และเอ็นไซม์ในซีรัมยางพารานี้มีแอกทิวิตีในการย่อยสูง
สุดที่อุณหภูมิ 45 °C โดยให้ผลิตภัณฑ์เป็นน้ำตาล GlcNAc และน้ำตาล (GlcNAc)₂ ด้วยอัตราส่วน
โมลผลิตภัณฑ์ ((GlcNAc)₂/GlcNAc) ประมาณ 2:1 และเมื่อย่อยไคติน 300 มิลลิกรัม จะได้น้ำตาล
GlcNAc 39 มิลลิกรัมและน้ำตาล (GlcNAc)₂ 108 มิลลิกรัม คิดเป็น 11.6 เปอร์เซ็นต์ และ 35.8
เปอร์เซ็นต์ ตามลำดับด้วยการวิเคราะห์ด้วย HPLC ในเวลา 8 วันทำการย่อยด้วยเอ็นไซม์ 64 มิลลิ
วินิต ที่สภาวะเหมาะสม เทคนิคไดอะไลซิสช่วยแยกผลิตภัณฑ์ออกจากไคตินและเอนไซม์ได้ง่าย
แต่ประสิทธิภาพของเอนไซม์กลับลดลง น้ำตาล (GlcNAc)₂ ถูกทำให้บริสุทธิ์ด้วย gel-filtration
chromatography และพบว่า น้ำตาล (GlcNAc)₂ ได้คืนมา 92 เปอร์เซ็นต์ ด้วยความบริสุทธิ์ 36
เปอร์เซ็นต์โดยน้ำหนัก เทคนิคการผสมเอนไซม์ได้ถูกใช้สำหรับการผลิต GlcNAc จากไคติน ซึ่ง
ปฏิกิริยานี้ให้ 52 เปอร์เซ็นต์ของ GlcNAc วิเคราะห์โดย HPLC ในวันที่ 4 และ ซีรัมให้ผลิตภัณฑ์
เป็นไคโทซานมวลโมเลกุลต่ำ 5.4×10^4 - 1.5×10^5 แทนที่ไคโทโอลิโกแซ็กคาไรด์เมื่อย่อยด้วยไคโท
ซาน ดังนั้นซีรัมจึงมีศักยภาพที่จะใช้ในการผลิตน้ำตาล GlcNAc และ (GlcNAc)₂ และไคโทซาน
มวลโมเลกุลต่ำ

ภาควิชา.....เคมี.....ลายมือชื่อนิสิต..... 10กมล คล้ายเกา
สาขาวิชา.....เคมี.....ลายมือชื่ออาจารย์ที่ปรึกษา.....
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AKAMOL KLAIKHERD: PREPARATION OF *N*-ACETYL-D-GLUCOSAMINE AND CHITOLIGOSACCHARIDE BY ENZYMATIC HYDROLYSIS OF CHITIN AND CHITOSAN WITH SERUM FROM PARA RUBBER. THESIS ADVISOR: ~~ASST.~~ PROF. MONGKOL SUKWATTANASINITT, Ph.D.; THESIS CO-ADVISOR: ASSOC. PROF. SUPASON WANICHWEACHARUNGRUANG, Ph.D., 127 pp. ISBN 974-17-2541-8.

Serum fraction of para rubber (*Hevea brasiliensis*) obtained from the process of concentrated latex preparation is known to contain an endo-chitinolytic enzyme, Hevamine. This work presents an investigation of a potential utilization of the serum for the production of *N*-acetyl-D-glucosamine (GlcNAc) and *N,N'*-diacetylchitobiose ((GlcNAc)₂) from β -chitin. The rubber serum contained 6 mg/mL of protein with chitinolytic activity of 18 mU per milligram of protein. The optimum ratio of enzyme to chitin was found to be 0.22 mU/mg with optimum substrate concentration at 60 mg/mL. The optimum pH range was 2-4 and the optimum temperature was 45 °C where the reaction produced both (GlcNAc)₂ and GlcNAc with the product mole ratio ((GlcNAc)₂/GlcNAc) approximately 2:1. The hydrolysis of 300 mg of chitin yielded 39 mg of GlcNAc and 108 mg of (GlcNAc)₂ corresponding to HPLC yield of 11.6% GlcNAc and 35.8% (GlcNAc)₂ within 8 days when 64 mU of the enzyme was used at the optimum condition. Dialysis technique offered convenient separation of the GlcNAc and (GlcNAc)₂ products from the starting chitin and enzymes but reduced the enzyme efficiency. Partial purification of (GlcNAc)₂ was achieved by gel filtration chromatography. (GlcNAc)₂ was recovered in 92% with 36% (w/w) purity. A technique of enzyme combination was used for production of GlcNAc from chitin. This hydrolysis showed an HPLC yield of 52% GlcNAc in 4 days. The serum *Hb* gave low molecular weight chitosan with M_w 5.4×10^4 - 1.5×10^5 rather than the expected chitooligosaccharide (GlcNAc)₂ - (GlcNAc)₇ when hydrolyzed with chitosan. The serum thus has potential use for low cost production of GlcNAc and (GlcNAc)₂ and low molecular weight chitosan.

Department.....Chemistry.....Student's signature.....*Akamol Klaiherd*
 Field of study.....Chemistry.....Advisor's signature.....*Supason Wanichweacharungruang*
 Academic year.....2002.....Co-advisor's signature.....*Supason Wanichweacharungruang*

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List of Abbreviations

cm	centimeter	min	minute
°C	degree celsius	mL	milliliter (s)
DI-water	deionized water	mM	millimolar
DOC	sodium deoxycholate	mU	milliunit
g	gram (s)	M _w	molecular weight
GlcNAc	<i>N</i> -acetyl-D-glucosamine	ppm	part per million
(GlcNAc) ₂	<i>N,N'</i> -diacetylchitobiose	PBS	phosphate buffer saline
(GlcNAc) ₃	<i>N,N',N''</i> -triacetyl- chitotriose	PTA	phosphotungstic acid
(GlcNAc) ₄	<i>N,N',N'',N'''</i> -tetraacetyl- chitotetraose	sec	second
GlcN	D-glucosamine	TCA	trichloroacetic acid
GFC	gel filtration chromatography	U	unit
GPC	gel permeation chromatography	Å	angstrom
HPLC	high performance liquid chromatography	α	alpha
mg	milligram	β	beta
M	molar	γ	gamma
		μL	microliter
		%	percent
		%DA	percent degree of acetylation

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