

ผลของตัวทำละลายต่อการเปลี่ยนแปลง โครงรูปและการซึมผ่านผิวหนังสัตว์ทดลอง  
แบบนอกร่างของเปปไทด์ต้นแบบไลโซไซม์



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SOLVENT EFFECT ON THE CONFORMATIONAL MODIFICATION AND  
IN VITRO ANIMAL SKIN PERMEATION OF LYSOZYME MODEL PEPTIDE

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 สัตว์ทดลองแบบนอกร่างของเปปไทด์ต้นแบบไลโซไซม์ (SOLVENT EFFECT ON  
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การศึกษานี้มีจุดประสงค์ที่จะจำแนกสถานะโมลเทนกลอบูล (Molten globule) หรือสถานะที่มีการม้วนพับของ  
 โปรตีนบางส่วนของไลโซไซม์และการซึมผ่านผิวหนังสัตว์ทดลองแบบนอกร่าง คุณลักษณะของสถานะโมลเทนกลอบูล  
 คือ การสูญเสียโครงสร้างตติยภูมิโดยโครงสร้างทุติยภูมิที่คล้ายโครงสร้างธรรมชาติยังคงอยู่และเกิดการอัดแน่น การศึกษา  
 นี้ทำเพื่อที่จะพิจารณาผลของสภาวะแวดล้อมที่เป็นสารละลายผสมระหว่างน้ำและเอทานอล สภาวะแวดล้อมที่เป็นกรด  
 และสภาวะแวดล้อมผสมต่อการเปลี่ยนแปลงโครงรูปของไลโซไซม์ สถานะโมลเทนกลอบูลเกิดขึ้นในสภาวะที่ประกอบด้วย  
 ไลโซไซม์ในสารละลายผสมระหว่างน้ำกับเอทานอลในร้อยละ 80 โดยปริมาตรของเอทานอล [L(80)(0)] หรือใน  
 ร้อยละ 35 โดยปริมาตร หรือ ร้อยละ 40 โดยปริมาตรของเอทานอลในสารละลายผสมระหว่างน้ำและเอทานอลใน 20  
 มิลลิโมลาร์ [L(35)(20) หรือ L(40)(20) ตามลำดับ] ยิ่งไปกว่านั้นไลโซไซม์ที่ถูกเปลี่ยนแปลงโครงสร้างในสภาวะที่ได้  
 กล่าวมาข้างต้นจะจับกับ 1-อนิลิโน-8-แนฟทาลีนซัลโฟเนตแอนไอออน (ANS) ได้ดีเยี่ยมแสดงถึงสถานะโมลเทนกลอบูล  
 สถานะนี้สามารถกลับสู่โครงรูปธรรมชาติได้เมื่อเจือจางด้วยน้ำยี่สิบเท่าของปริมาตรเริ่มต้น อย่างไรก็ตามสภาวะแวดล้อม  
 ที่เป็นกรดเดี่ยวๆไม่สามารถเหนี่ยวนำไลโซไซม์ให้เกิดสถานะโมลเทนกลอบูลได้ ไลโซไซม์ที่ละลายใน 4 นอร์มอล  
 ไฮโดรคลอริก ข้ามคืนนำไปสู่การตกตะกอนโปรตีนขณะที่โครงสร้างไลโซไซม์ถูกทำลายอย่างสมบูรณ์ใน 6 นอร์มอล  
 ไฮโดรคลอริก สารละลายของ L(80)(0) และ L(35)(20) ถูกเลือกเป็นสารละลายที่เหนี่ยวนำไลโซไซม์ให้เป็นสถานะ  
 โมลเทนกลอบูลเพื่อการทดลองของการซึมผ่านโดยใช้ผิวหนังของหนูเป็นผิวหนังสัตว์ทดลองต้นแบบแบบนอกร่างการ  
 ศึกษาผลของกระสวยต่อความสมบูรณ์ของผิวหนังพิจารณาโดยใช้โพรพาราโนลไฮโดรคลอไรด์เป็นตัวบ่งชี้ แม้ว่า  
 กระสวยเหล่านี้ไม่ได้เป็นสาเหตุของการรั่วของผิวหนังต้นแบบแต่มีแนวโน้มทำให้การซึมผ่านของโพรพาราโนลไฮโดร  
 คลอไรด์ลดลง การพิจารณาผิวหนังของหนังก่อนหลังการสัมผัสกับเอทานอลโดยใช้กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด  
 ด้วยเทคนิคเยือกแข็งแสดงการถูกกำจัดน้ำออกของหนังและไขมันถูกสกัดออกมาจากหนังต้นแบบ ขณะที่การสัมผัสของ  
 หนังกับสภาวะแวดล้อมผสมระหว่างน้ำและเอทานอลกับกรดแสดงให้เห็นว่าการบวมและการเปลี่ยนแปลงในโครงสร้าง  
 ของผิว การพิจารณาปริมาณของไลโซไซม์ที่เหลือในโดเนอรัคคอมพาร์ตเมนต์ (donor compartment) แสดงให้เห็นว่า  
 ไลโซไซม์จาก L(80)(0) และ L(35)(20) ลดลงจากโดเนอรัคคอมพาร์ตเมนต์เร็วกว่าจาก L(0)(0) และ L(35)(0) ซึ่งควรจะหา  
 สาเหตุที่ชัดเจนต่อไป อย่างไรก็ตามเหตุผลหนึ่งที่เป็นไปได้คือสถานะของไลโซไซม์ที่เป็นโมลเทนกลอบูลมีคุณลักษณะ  
 ที่ไม่ชอบน้ำสูงซึ่งจึงทำให้ซึมผ่านผิวหนังได้ดีขึ้น

ภาควิชา ..... เกษัตริย์อุตสาหกรรม.....  
 สาขาวิชา..... เกษัตริย์อุตสาหกรรม.....  
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ลายมือชื่อนิติ..... วิริยาพร ศิริกุล.....  
 ลายมือชื่ออาจารย์ที่ปรึกษา.....  
 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

##4576600733 MAJOR: MANUFACTURING PHARMACY

KEYWORD: LYSOZYME/ CIRCULAR DICHROISM/ FLUOROMETRY/ PIG'S EAR SKIN/ ETHANOL/ PERMEATION

WIRIYAPORN SIRIKUN: SOLVENT EFFECT ON THE CONFORMATIONAL MODIFICATION AND IN VITRO ANIMAL SKIN PERMEATION OF LYSOZYME MODEL PEPTIDE. THESIS ADVISOR: NARUEPORN SUTANTHAVIBUL, Ph.D., THESIS CO-ADVISOR: NONTIMA VARDHANABHUTI, Ph.D., 133 PP. ISBN 974-53-2758-1.

The present study was aimed to characterize lysozyme in its molten globule (MG) or partially folded state and to study the in vitro animal skin penetration. The characteristics of MG state are represented as a loss in the tertiary structure, while retaining the native-like secondary structure and an evidence of compactness. This study was done to determine the effects of aqueous-ethanolic environment, acidic environment and the combined environment on the conformational modification of lysozyme. The evidence of MG state occurred in the conditions which composed of lysozyme in 80% v/v of ethanol in the aqueous-ethanolic solution [L(80)(0)] or 35% v/v or 40% v/v of ethanol in the aqueous-ethanolic solution in 20 mM of HCl acid [L(35)(20) or L(40)(20), respectively]. Moreover, the modified lysozyme in above conditions was shown to be efficiently bound to 1-anilino-8-naphthalene sulfonate anion (ANS) which signified MG state. This state could be reversed to native conformation when diluted with water at twenty times its initial volume. Nevertheless, the acidic environment alone could not induce lysozyme to MG state. Lysozyme dissolved in 4 N HCl overnight led to protein aggregation while the structure of lysozyme was completely destroyed in 6 N HCl. The solutions of L(80)(0) and L(35)(20) were selected as solvents to induce lysozyme to MG state for the penetration experiment using pig's ear skin as an in vitro model skin. The effects of vehicles on the integrity of the skin were determined using hydrophilic propranolol HCl as a marker. Although these vehicles did not cause leakage of model skin, they decreased the penetration of propranolol HCl. Determination of the skin surface after ethanol exposure using Cryo-SEM showed dehydration and lipid extraction of the skin. Exposure of the skin to the combined environment, where both aqueous-ethanol and acid were presented, demonstrated swelling and changes in the skin structure on the model skin. The determination of the amount of lysozyme remaining in the donor compartment showed that the lysozyme from L(80)(0) and L(35)(20) disappeared from the donor side faster than that from L(0)(0) and L(35)(0). The reason for this finding still needed further investigation. However, penetration of lysozyme in MG state with increasing hydrophobic character was one of the possibilities.

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Field of study.....Industrial Pharmacy.....Advisor's signature..M. Sutanthavibul

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## ABBREVIATIONS

°C	=	degree Celsius
CD	=	circular dichroism
cm	=	centimeter
DSC	=	differential scanning calorimetry
et al.	=	<i>et alii</i> , 'and others'
Ex	=	excitation
FT-IR	=	fourier-transform infrared
g	=	gram
h	=	hour
kDa	=	kilo dalton
l	=	liter
MG	=	molten globule
mg	=	milligram
mdeg	=	milidegree
min	=	minute
ml	=	milliliter
mM	=	millimolar (concentration)
mol/l	=	molar per liter
N	=	normal (concentration)
nm	=	nanometer
NMR	=	nuclear magnetic resonance
pH	=	the negative logarithm of the hydrogen concentration
S.D.	=	standard deviation
SDS-PAGE	=	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEM	=	scanning electron microscope
sec	=	second
μl	=	microliter
v/v	=	volume by volume
w/v	=	weight by volume