Lysozyme Enzyme

Contents

- Introduction

Lysozyme is 129 amino acid residues enzyme .
It is a basic bacteriolytic Protein that hydrolyzes peptidoglycan, glycosidic bonds and are widely distributed in nature.
(EC 3.2.1.17), hydrolase which catalyzes hydrolysis of 1,4-beta-linkages between Nacetylmuramic acid and N-acetyl-D-glucosamine residues in peptidoglycan and between N-acetylD-glucosamine residues in chitodextrins.
Molecular weight of Lysozyme is an approximately 14.7 kDa .

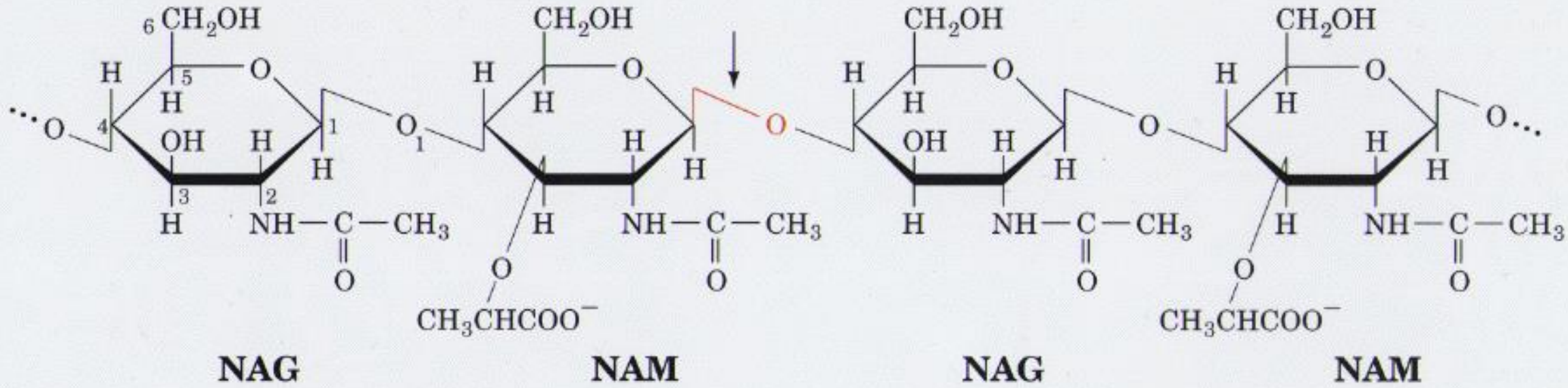
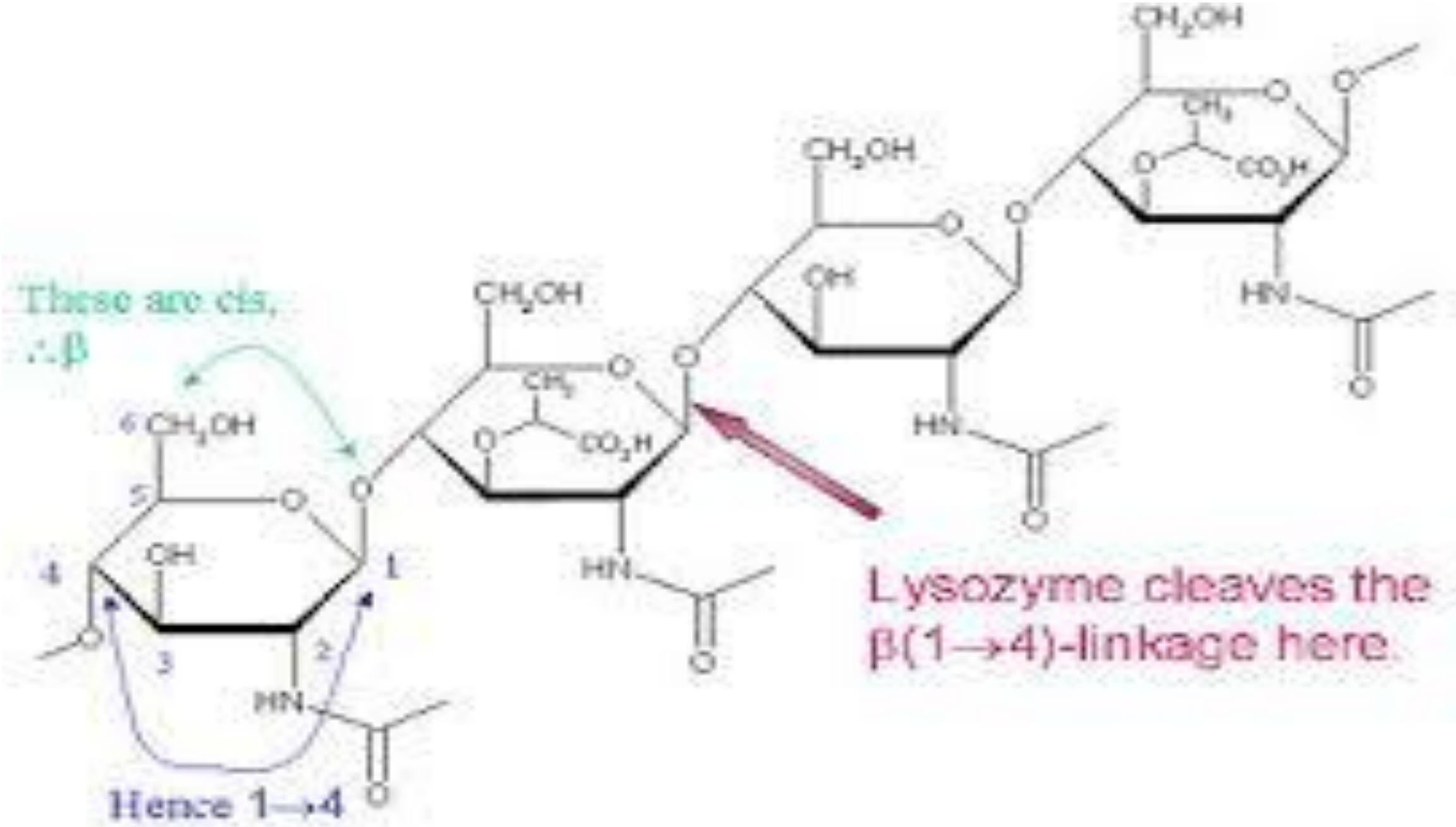


Figure 14-8. The polysaccharide substrate of lysozyme.


These are cis,
∴β
Lysozyme cleaves the
β(1→4)-linkage here.
Hence 1→4

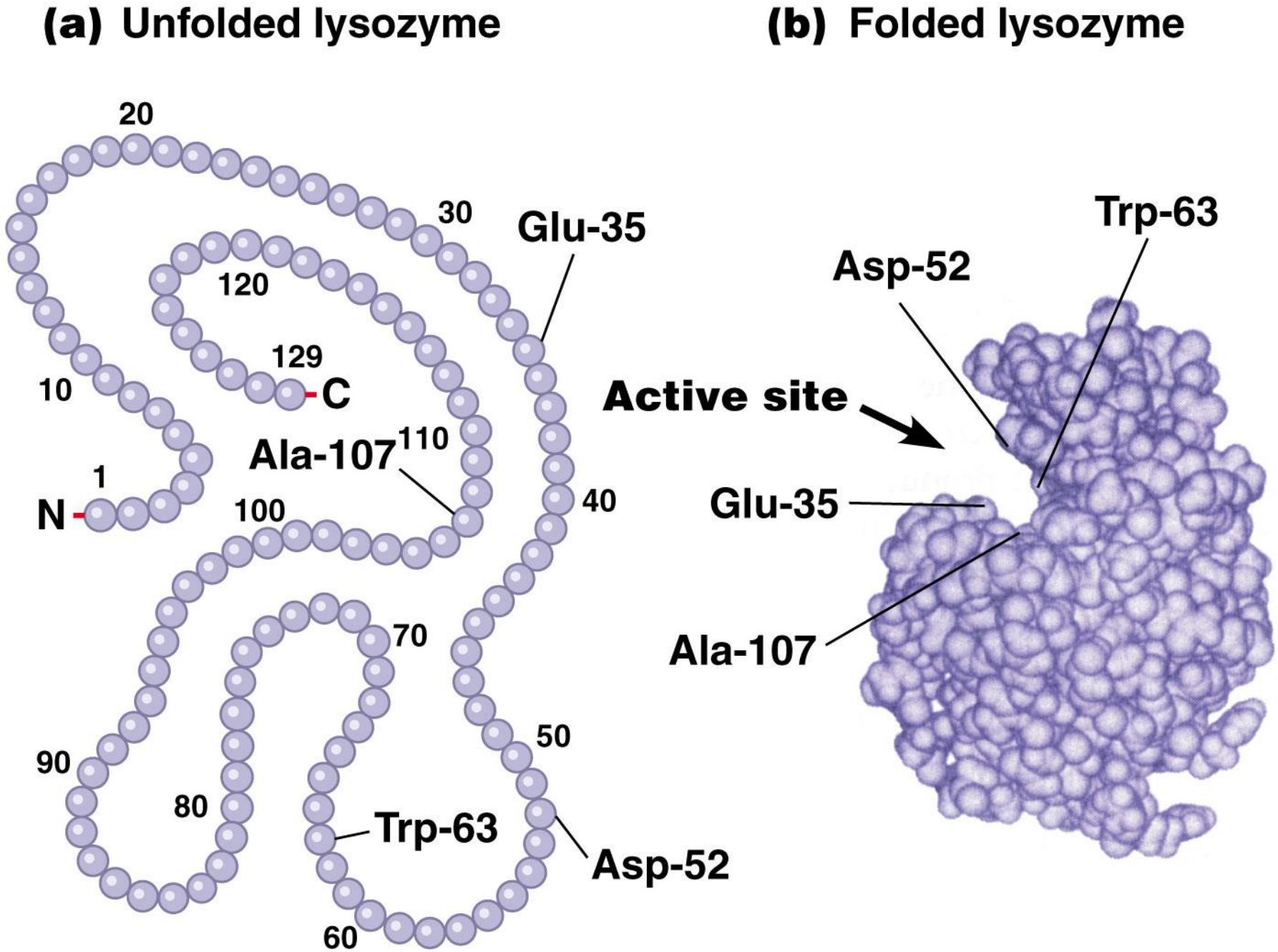
- History
- Laschtschenko first discovered Lysozyme in 1909, when he first observed the antibacterial property of hen egg whites.
- Lysozyme was discovered in 1922 by Alexander Fleming on a remarkable becteriolytic element found in tissues and secretion.
- In 1965 the structure of Lysozyme was solved by XRay analysis with 2 angstrom resolution by David Chilton Phillips.

- Functions
- As an antibacterial agent by catalyzing the hydrolysis of specific glycosidic linkages in peptidoglycan and chitin, breaking down some bacterial cell walls.
- hydrolyze the ß(1-4) glycosidic bond between residues of N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) in certain polysaccharides.
- Acts as a mild antiseptic.
- As a model protein for studying on structure and function of protein.

- Properties
- Lysozyme has the characters of a ferment. The rapidity of its action increases up to 60° C, but at temperatures over 65° C. it is destroyed more or less rapidly.
- It acts best in a neutral medium.
- Peptic or tryptic digestion does not destroy Lysozyme.
- Stability—When kept dry, Lysozyme can be preserved for a long time. It was noted that commercial dried egg albumen was very rich in Lysozyme .

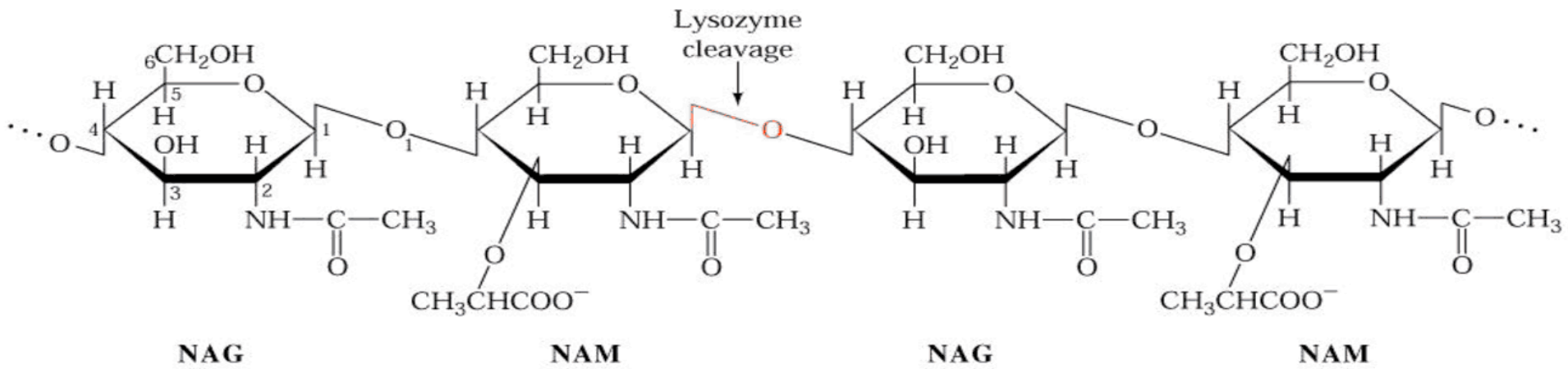
- Structure

- Lysozyme is a compact protein of 129 amino acids which folds into a compact globular structure.
- It has an alpha+beta fold, consisting of five to seven alpha helices and a three-stranded antiparallel beta sheet. The enzyme is approximately ellipsoidal in shape, with a large cleft in one side forming the active site.
- It comprises of 2 domains joined by a long Alpha helix between which lies the active site for antimicrobial activity;
- N-terminal domain(residues 40-88) has some helices and Beta parallel sheets.
- The Second domain (1-39 and 89-129) has mostly Alpha helical structure.
- 4 Sulphide bonds in locations between Cys 6-Cys127,Cys30Cys115,Cys 64-Cys 80 and Cys 76-Cys 94 lend stability and unusual compaction.



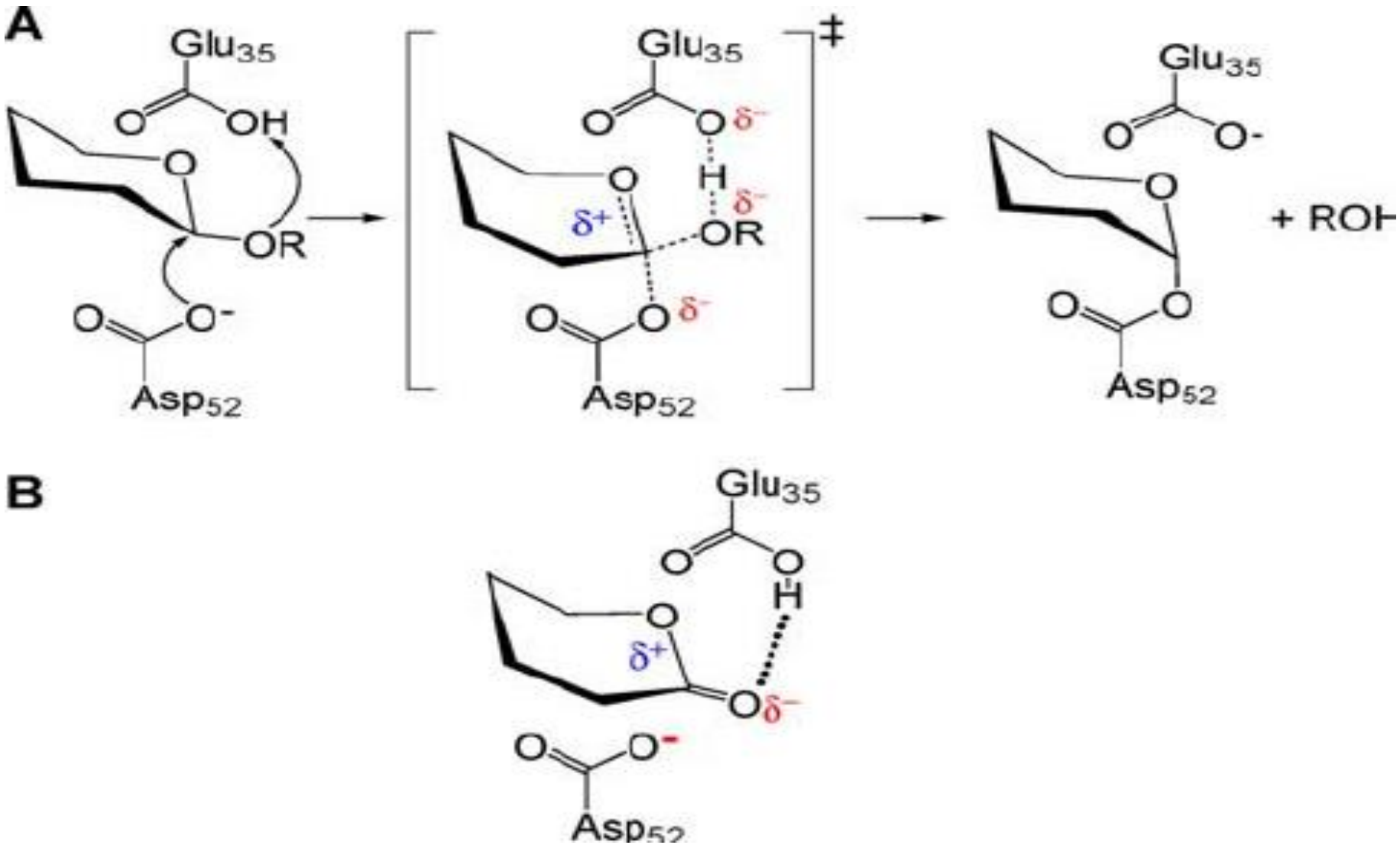
Mechanism of lysozyme

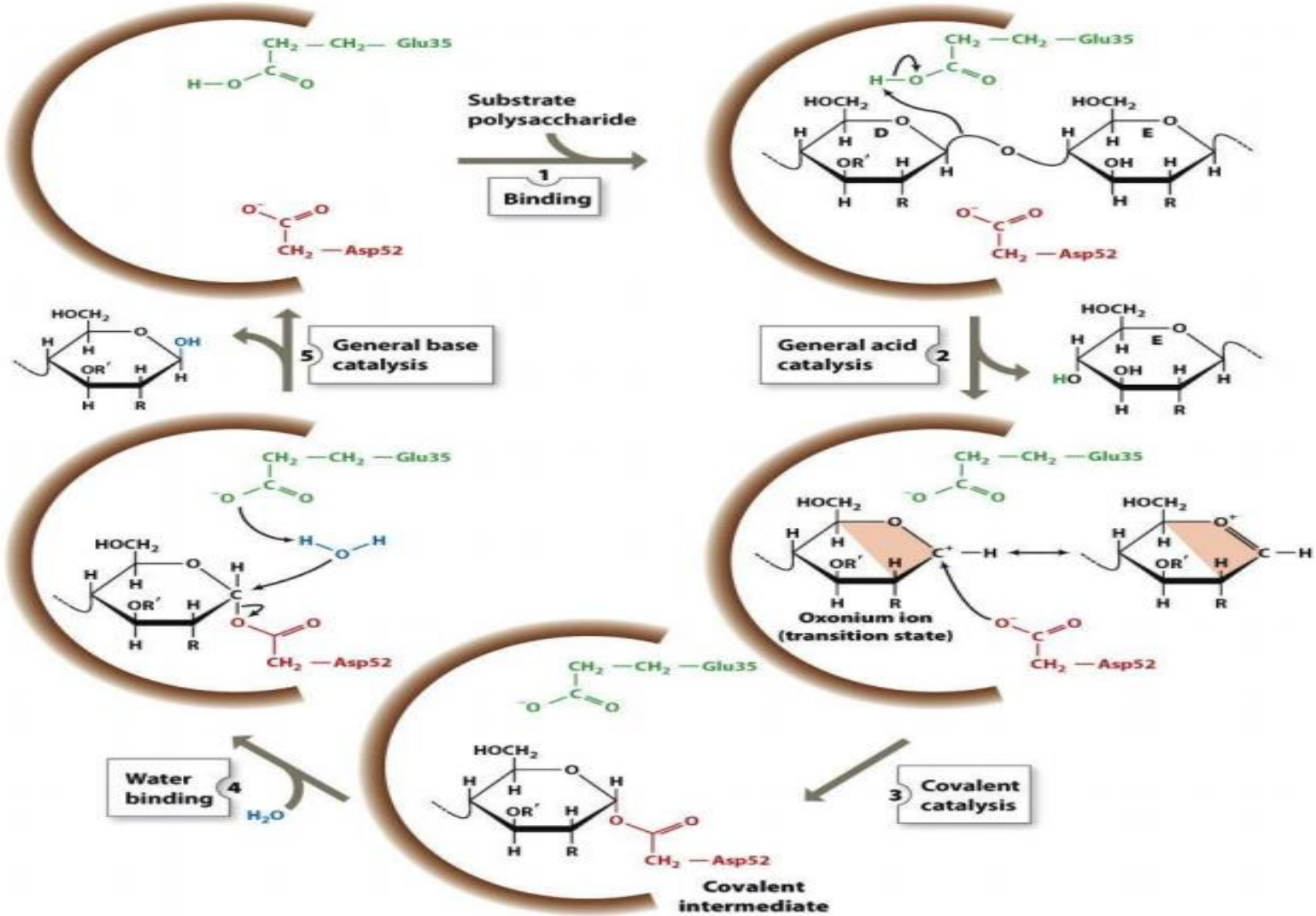
Lysozyme digests bacterial cell walls by breaking β(1- 4) glycosidic bonds between (N- acetylmuramic acid (NAM) and N-acetylglucosamine (NAG)



- Catalysis
- For catalysis to occur, (NAG-NAM)3 binds to the active site with each sugar in the chair conformation except the fourth which is distorted to a half chair form, which labilizes the glycosidic link between the 4th and 5th sugars.
- if the sugars that fit into the binding site are labeled A-F, then because of the bulky lactyl substituent on the NAM, residues C and E can not be NAM, which suggests that B, D and F must be NAM residues.
- Cleavage occurs between residues D and E. Catalysis by the enzyme involves Glu 35 and Asp 52 which are in the active site.
- Asp 52 is surrounded by polar groups but Glu 35 is in a hydrophobic environment.
- This should increase the apparent pKa of Glu 35, making it less likely to donate a proton and acquire a negative charge at low pH values, making it a better general acid at higher pH values. The general mechanism appears to involve.

- . Lysozyme attaches to a bacterial cell wall by binding to a hexasaccharide unit. The D residue is distorted towards the half-chair.
- 2. Glu 35 transfers its proton to the O1 of the D ring (general acid catalysis) C1-O1 bond is cleaved generating an oxonium ion at C1.
- 3. Asp 52 stabilizes the oxonium ion through charge-charge interactions. The carboxylate can not form a covalent bond because distances are too great. Reaction via a SN2 mechanism with transient formation of a C --O bond to the enzyme.
- 4. E ring group is released from the enzyme yielding a glycosyl-enzyme intermediate which adds water to reverse the chemistry and reprotonate Glu 35.

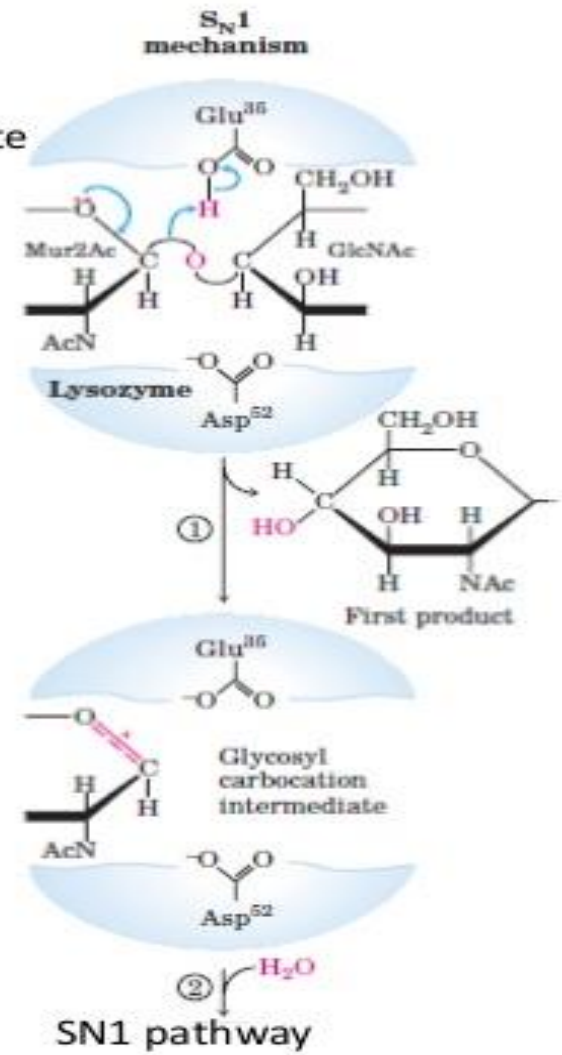

A
Glu35
OH
O
OR
O-
Asp52
‡
δ-
δ+
H
+ ROH
B


Substrate polysaccharide
1 Binding
2 General acid catalysis
3 Covalent catalysis
4 Water binding
5 General base catalysis
Glu35
Asp52
Oxonium ion (transition state)
Covalent intermediate

Lysozyme Mechanism

SN1 pathway

Peptidoglycan binds in the active site of lysozyme



- The key catalytic amino acid residues in the active site are **Glu35 and Asp52**.
- The reaction is a nucleophilic substitution, with --OH from water replacing the GlcNAc at C-1 of Mur2Ac.

In **SN1-type mechanism**, the GlcNAc initially dissociates in step 1 to leave behind a glycosyl cation (a carbocation) intermediate.

In this mechanism, the departing GlcNAc is protonated by general acid catalysis by Glu35, located in a hydrophobic pocket that gives its carboxyl group an unusually high p*K*a.