Lysozyme is 129 aminoacid residues enzyme (EC 3.2.1.17) which catalyzes hydrolysis of 1,4-beta-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in peptidoglycan and between N-acetyl-D-glucosamine residues in chitodextrins. Molecular weight of Lysozyme is an approximately 14.7 kDa. Alternative name for Lysozyme are 1,4-N-acetylmuramidase, L-7001, N,O-diacetylmuramidase, PR1-Lysozyme, Globulin G1, Globulin G, Lysozyme g, Mucopeptide N-acetylmuramoylhydrolase, Mucopeptide glucohydrolase and Muramidase. This catalytic activity is non-specifically targeted to the bacterial cell membranes and related with general non-specific organism defence.

Lysozyme is present in the mucosal secretion such as saliva and tears. In high concentration, about 3% from all proteins, Lysozyme is present in chicken egg-white. This enzyme is only effective against Gram positive bacterial cells. Gram negative bacteria and yeast are completely resistant to lysing by it.

Historically, Lysozyme was discovered in 1922 by Alexander Fleming (Fleming A. (1922) On a remarkable bacteriolytic element found in tissues and secretion. Proc Roy Soc Ser B, 93, 306-317.). This enzyme was discovered by accident, which was happen in the Fleming's lab. The nasal drippings were accidentally occurring in the petre dish with bacterial culture and these cells were lysed. This phenomenon was carefully investigated and the main acting enzyme was identified as Lysozyme.

In 1965 the structure of Lysozyme was solved by X-Ray analysis with 2 angstrom resolution by David Chilton Phillips. For many years Lysozyme was the best object for X-Ray analysis due to many unique properties of this enzyme. First of all Lysozyme is easy to purify from egg-white. Secondly, this protein is very easy to crystallize, which is not the case for most of the other proteins. This feature of Lysozyme is widely used for it purification. And finally, crystals of Lysozyme diffract X-Ray beam to a very high resolution, currently the highest resolution structure, presented in Protein Data Bank, was solved at resolution 0.94 Angstrom.

In viruses (or bacteriophages), Lysozyme is used as an agent to break into the host bacterial cell. Lysozyme from the tail of the virus (or bacteriophage) destroys the peptidoglycan bacterial cell wall and then virus can injects its DNA. After multiplication in bacteria, many Lysozyme molecules are created to lyse the bacterial cell wall and release new viruses.
Hydrolysis of 1,4-beta-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine and between N-acetyl-D-glucosamine by Lysozyme reaction, Enzyme EC 3.2.1.17

Lysozyme belongs to the **hydrolases** (EC 3.-.-.-) enzymatic class. The hydrolases catalyze the hydrolysis or hydrolytic cleavage of a chemical bond by reaction: \( A - B + H_2O \rightarrow A - OH + B - H \). This class of enzymes is usually classified by nature of the **hydrolysed bond**, then by chemical nature of the **substrate**, and finally by the **enzyme**. Despite systematic name for hydrolases always include **hydrolase**, the recommended name is formed by the name of the substrate with the suffix **-ase**.

Within the class of hydrolases, Lysozyme belongs to the **Glycosylases** family (EC 3.2.-.-.). Lysozyme reaction is the hydrolysis of the beta (1-4) glycosidic bond between N-acetylglucosamine sugar (NAG) and N-acetylmuramic acid sugar (NAM) and therefore it is possible classify it as **Glycosidases, i.e. enzymes hydrolyzing O- and S-glycosyl** (EC 3.2.1.-) with number 17 (EC 3.2.1.17) in this group.

Lysozyme reaction is hydrolysis of the beta (1-4) glycosidic bond between N-acetylglucosamine sugar (NAG) and N-acetylmuramic acid sugar (NAM). These bonds are circled on the following figure. This reaction is take place in a long deep cleft, which contains the active site of Lysozyme (residues Glu35 and Asp52 for chicken egg white Lysozyme enzyme). This cleft is a very specific active site, which can bind only six sugar rings from a polysaccharide chain and hydrolyze them into a disaccharide and a tetrasaccharide subunit.

### Lysozyme substrate

![Lysozyme substrate](image)

**Description**

The beta (1-4) glycosidic bond between N-acetylglucosamine sugar (NAG) and N-acetylmuramic acid sugar (NAM) to be hydrolysed during the Lysozyme reaction are circled.

Lysozyme

I.U.B.: 3.2.1.17
Mucopeptide \( N\)-acetylmuramolylhydrolase

Lysozyme (muramidase) hydrolyzes preferentially the \( \beta\)-1,4 glucosidic linkages between \( N\)-acetylmuramic acid and \( N\)-acetylglucosamine which occur in the mucopeptide cell wall structure of certain microorganisms, such as *Micrococcus lysodeikticus*. A somewhat more limited activity is exhibited towards chitin oligomers (Holler *et al.* 1975a and b).

Lysozyme is of widespread distribution in animals and plants. That which has been most extensively studied is from hen egg white (lysozyme "c"). A second avian egg white lysozyme is that of the domestic goose designated lysozyme "g" (Prager *et al.* 1974). Lysozyme is also found in mammalian secretions and tissues, saliva, tears, milk, cervical mucus, leucocytes, kidneys, etc.

Human lysozyme from urine has been crystallized by Osserman (1967) and extensively investigated (Osserman *et al.* 1974). It has been indicated (Osserman *et al.* 1973) that lysozyme may be the mediator in the anti-tumor function of macrophages which, it has been shown, secrete the enzyme (Gordon *et al.* 1974). See also Asdourian *et al.* (1975). There is evidence that cartilage lysozyme has a role in cartilage calcification (Kuettner *et al.* 1974).

There has been interest in lysozyme as a "natural" antibiotic and as an aid in the diagnosis of disease (Glynn 1968; Pruzanski and Saito 1969). Elevated levels of serum and urinary lysozyme are present in monocytic and mono-myelocytic leukemia (Osserman and Lawlor 1966; Brieree *et al.* 1974). Presence of the enzyme in cerebrospinal fluid is indicative of tumor of the central nervous system (Newman *et al.* 1974). Hankiewicz and Swiervzek (1974) report that normally lysozyme activity is practically absent from urine, bile and spinal fluid.

Plant lysozyme is found in ficus and papa latex, and chemically is distinct from the egg white enzyme (Meyer *et al.* 1946). Jollés and Jollés (1975) have reported on lysozyme from *Asterias rubens*. A polypeptide with lysozyme activity has been synthesized (Sharp *et al.* 1973).

The enzyme has been used for lysing *E. coli* and *Streptomycetes* for extraction purposes (Haas and Dowding 1975) such as extracting group specific antigen (Watson *et al.* 1975). It would appear that lysozyme may act as a germinative agent of bacterial spores (Ando 1975; Duncan *et al.* 1972).

Considerable physicochemical information is available for lysozyme. Structure-function relationships are thoroughly reviewed by Imoto *et al.* (1972) and Phillips (1972). Jollés (1969) and Chipman and Sharon (1969) have provided descriptions of the enzyme and its catalytic mechanism.

**Characteristics of Lysozyme from Chicken Egg White:**

**Molecular weight:** 14,388 (Jollés 1969).

**Composition:** Amino acid sequence is reported by Jollés *et al.* (1963) and Canfield (1963). Tertiary structure is treated comprehensively by Imoto *et al.* (1972) and Warme and Scheraga (1974). Other reports on molecular properties are by Baldo *et al.* (1975),
Formoso and Forster (1975), Halford (1975), Hsi and Bryant (1975), Kuramitsu et al. (1975), Atkinson and Bruce (1974), Bertiou and Jollès (1974), Holler et al. (1974), and MatthysSENS and Kanarek (1974). Atassi et al. (1974) describe the binding site as a cleft across one side of the molecule that can accommodate six b(1—>4) less linked units of 2-acetamido-2-deoxy-D-glucopyranose.

**Optimum pH:** 9.2 (Davies et al. 1969).

**Extinction coefficient:** $\varepsilon_{450} = 26.4$ (Aune and Tanford 1969).

**Isoelectric point:** $\pi = 11.0$ (Alderton et al. 1945).

**Specificity:** Egg white and comparative lysozyme specificities are described by Hara and Matsushima (1967 and 1972).

**Inhibitors:** The enzyme is inhibited by surface-active reagents such as dodecyl sulfate, alcohols and fatty acids (Smith and Stoker 1949). Imidazole and indole derivatives are inhibitors via formation of change-transfer complexes (Shinitzky et al. 1966; Swan 1972).

**Stability:** Lysozyme stored as a dry lyophilized or crystalline powder at 2 - 8°C is stable for years. Solutions at pH 4-5 are stable for several weeks refrigerated and for days at ambient temperatures.

Mintz et al. (1975) have described a sensitive fluorimetric assay.

**Lysozyme Assay**

**Method:** The rate of lysis of *Micrococcus lysodeikticus* is determined as suggested by Shugar (1952). One unit is equal to a decrease in turbidity of 0.001 per minute at 450 nm at pH 7.0 and 25°C under the specified conditions. A wide range of activities are reported for pure lysozyme preparations under these conditions. The Worthington specific activity of 8,000 u/mg dw is equivalent to 50,000 u/mg dw claimed by other suppliers.

**Reagents**

- 0.1 M Potassium phosphate, pH 7.0
- *Micrococcus lysodeikticus* cells: Prepare by suspending 9 mg of dried *Micrococcus lysodeikticus* (Worthington code: ML) cells in 25 ml of 0.1 M potassium phosphate buffer, pH 7.0. Dilute to a final volume of 30 ml with the same buffer.

**Enzyme**

Dissolve the enzyme at a concentration of one mg/ml in cold reagent grade water. Keep cool until assay. Immediately prior to assay, dilute to a concentration of 150-500 units/ml with reagent grade water. (The rate should fall between 0.015-0.040 $\Delta A_{450}$/minute).
**Procedure**

Adjust spectrophotometer to 450 nm and 25°C.

Pipette 2.9 ml of *Micrococcus lysodeikticus* cell suspension into a cuvette and incubate for 4-5 minutes in order to achieve temperature equilibration and to establish blank rate, if any. Add 0.1 ml of appropriately diluted enzyme to cuvette and record the change in $A_{450}$ per minute from the initial linear portion of the curve.

**Calculation**

\[
\text{Units} = \frac{\Delta A_{\text{min}} \times 1000}{\text{mg enzyme in reaction mixture}}
\]

\[
\text{mg/ml} = \Delta A_{\text{min}} \times 0.59
\]

References:

Information for this lab is from:

1. [http://lysozyme.co.uk/](http://lysozyme.co.uk/)