Faculty of Women for, Arts, Science, and Education

Scientific Publishing Unit

Journal of Scientific Research in Science

Biological Sciences

Volume 41 , Issue 2 , 2024

ISSN 2356-8372 (Online) \ ISSN 2356-8364 (print)

Journal of Scientific Research in Science 2024, 41, (2),1:17

Contents lists available at EKB

Journal of Scientific Research in Science

Journal homepage: https://jsrs.journals.ekb.eg/

Review

An overview on Hyaluronidase enzyme types, mode of action, assay and therapeutic applications

Mai A. Ebraheem[1*], Esmail M. El-Fakharany[2,3], Sherif Moussa Husseiny[1] and Fafy A. Mohammed[1]

[1]Botany Department, Faculty of women For Arts, Science and Education, Ain shams university, Egypt.
[2]Protein Research Department, Genetic Engineering and Biotechnology Research Institute GEPRI, City of Scientific Research and Technological Applications (SRTA-City), New Borg Al Arab 21934, Alexandria, Egypt.
[3]Pharmaceutical and Fermentation Industries Development Centre (PFIDC), City of Scientifc Research and Technological Applications (SRTA-City), New Borg Al Arab 21934, Alexandria, Egypt.

Abstract

Hyaluronidase is an enzyme of the current era. Not only because of its medical importance for cancer therapy but also because of its great importance in many fields. Hyaluronidases have substantial biological activity in the realm of medicine in addition to being useful enzymes for producing low molecular weight hyaluronic acid. It is employed clinically as a significant drug diffusion promoter to aid in the absorption of drug and localized hematoma or edema dissipation following trauma or surgery. Hyaluronidases made from animals have some drawbacks, including few resources, poor content, high cost, and the possibility of virus infection. Contrarily, hyaluronidase generated from microorganisms are more widely used due to their abundant source, straightforward fermentation procedure, low cost, high purity, and minimal environmental contamination. Microbial hyaluronidases are known as hyaluronate lyases Owing to the unsaturated double bonds they form at non-reducing ends by breaking down hyaluronic acid through the β-elimination reaction. *Streptococcus spp*, *Staphylococcus spp*, and other microbes have all been found to have hyaluronate lyases to date.

Keywords: Hyaluronidase**,** purification**,** mechanism of action**,** medical applications.

***Corresponding author**: **Mai A. Ebraheem,** Botany Department, Faculty of women For Arts, Science and Education, Ain shams university, Egypt.
Email: mai.abdelnaby@women.asu.edu.eg
(Received 29 Feb 2024, revised 18 Mar 2024, accepted 20 Mar 2024)
https://doi.org/10.21608/JSRS.2024.273369.1125

1. Introduction

Hyaluronidases are class of enzymes called glycosidase enzymes as they hydrolyze hyaluronic (HA) acid which is polysaccharide substrate **[1].** This enzyme is distributed in many parts of the body such as kidney and spleen and also body fluids such as blood, tears and seminal fluid **[2]**. Hyase enzyme is becoming more and more popular due to its significant applications in the commercial, physiological, biological, and medical commercial domains **[3]**. In the past, Medical hyase was originally derived from the testicles of sheep or cows and was used unpurified. Furthermore, the mammalian hyase that was produced in this manner had components that might trigger an immunological reaction and was not very pure. After that, microbial hyaluronidase was added to the processing sequence to lessen adverse effects, and purification of mammalian hyase was introduced as a subsequent step **[4].**

Because these enzymes help avoid problems from improper HA injection, remove HA nodules, and rectify unattractive HA overfilling, they are commonly used in aesthetic medicine **[5]**. For many years, spreading agents like as hyaluronidases have been used to encourage the subcutaneous diffusion of various substances **[6],** eliminate the cumulus-corona-oocyte complex that is created when sperm is injected intracytoplasmically **[7],** to stop tissue damage following several chemicals' extravasation **[8]**, for edema reduction **[9],** and for treatment of vitreous hemorrhage **[10]**.

In cancer, The frequency of CD44 receptors in a cell membrane is systematically increased. This receptor functions as a specific receptor for HA, which is known to stimulate the migration, metastasis, invasion, and spread of cancer cells. for this reason, Hyaluronidase acts as an anticancer by destroying HA in tissues and preventing the CD44–HA association. Hyase also has the ability to enhance the passage of antibiotics towards the synovial fluid.**[11].** Hyaluronidase can be obtained from different microrganisms such as Streptococcus, Streptomyces and Bacillus **[12, 13 ,14].** This review article spot light on the types of hyaluronidase, mode of action, purification and applications**.**

1.1. Nomenclature and types of hyaluronidase

There are six types of hyaluronidase can be recognized ;Hyaluronidases 1-4, PH-20, and Hyaluronidase P1 **[15]**. Along with its presence in serum and urine, hyaluronidase 1, a kind that is encoded by the hyaluronidase gene, is found in important organs such the liver and

kidney. It functions as a significant hyaluronidase in plasma and is activated at a pH that is acidic. Only high-molecular weight HA is broken down by hyaluronidase 2, which has less enzymatic activity than hyaluronidase 1. hyaluronidase 3 can be found in the testis and bone marrow **[16]** and has not yet been assigned a specific function **[17].** Human sperm and the inner crosomal membrane contain the enzyme testicular PH20 hyaluronidase, whose function is to break down HA in the ovum during fertilization **[18].**

1.2. Mode of action of hyaluronidases

Hyaluronidases were first divided by Meyer into three kinds based on their mode of action **[19]** into three groups based on variations in their catalytic mechanisms (Fig. 1). Hyaluronate 4-glycanohydrolases, the first type, are primarily found in venoms and vertebrates. (EC 3.2.1.35). These enzymes have the ability to break down HA and chondroitin sulphates to produce tetrasaccharides by breaking the β-1,4-glycosidic bond. **[20]**. Hyaluronate 3-glycanohydrolases (EC 3.2.1.36) are a second category of enzymes that primarily come from leeches. This type of hyaluronidase make hydrolysis to HA at the β-1,3-glycosidic bond resulting in tetra- and hexa-saccharides and glucuronic acid is created at the reducing end. Hyaluronate lyases (EC 4.2.2.1), the third group, are unique to microbes. Through the β-elimination reaction, they break down HA to release unsaturated disaccharides, such as 2-acetamido-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid), and products with unsaturated carbon-carbon bonds at the non-reducing end.-D-glucose as final pruduct. **[19]**.

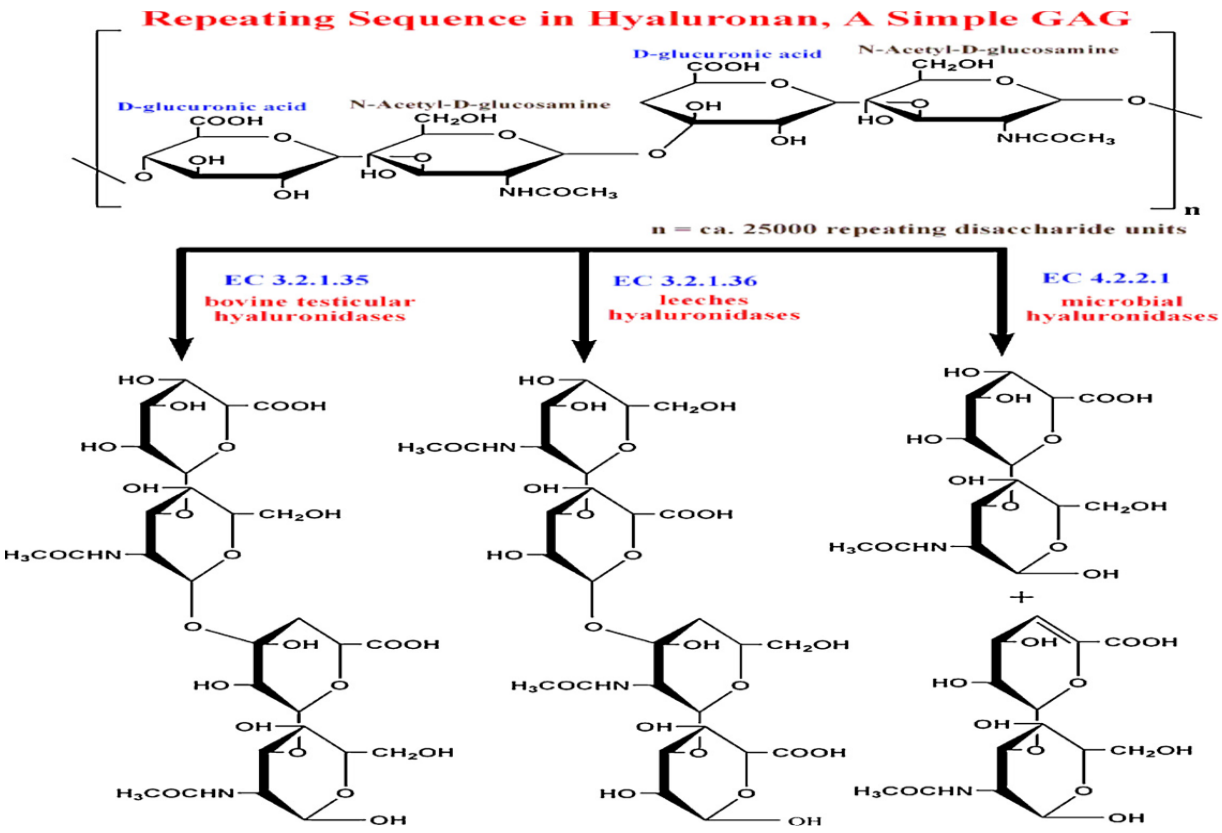

Fig. 1. Classification hyaluronidases based on mechanism of action

1.3. Sources of hyaluronidases

1.3.1. Hyase from venom

The venom of both vertebrates and invertebrates, such as spiders and bees, **[21]** lizards, scorpions **[22],** and snakes **[23]** has been used to describe and isolate hydrionoidases.. Hyase from bee venom (BVH) is the first eukaryotic enzyme to be generated recombinantly by cDNA cloning. **[24]**. The structure of Apis mellifera's recombinant hyaluronidase was established by Markovi´c and colleagues in 2000. **[25].**

1.3.2. Mammalian hyaluronidases

1.3.2.1. Bovine testicular hyase (BTH)

Most hyaluronidases that are available commercially are derived from mammals, with the most commonly used kind being hyaluronidase that is isolated from the testes of cows. The β-1,4 N-acethylhexosaminide linkages are broken by BTH, causing HA to break down into (GlcUA-GlcNAc) and (GlcUA-GlcNAc).

1.3.2.2. Hyases from human

Six hyaluronidase genes—Hyal-1, Hyal-2, Hyal-3, Hyal-4, and PH-20 are identified in the human genome. Mammals' liver, kidney, spleen, and heart all contain large amounts of hyal-1, which is also present in lysosomes. Additionally broadly distributed in the human body, hyal-2 attaches itself to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor [16]**.** Hyal-1 and Hyal-2 cooperate when the human body breaks down HA polymers into roughly 20 kDa pieces, which are subsequently broken down into tetrasaccharides by Hyal-1 **[4].** Nearly all mammals include PH-20 which is known as an adhesion molecule found in the sperm, a group of single-chain proteins with GPI anchors, however species-specific sequences vary. In contrast to Hyal-1 and Hyal-2, human PH-20 is mostly expressed in the testes, while it is also found in the epididymis, female genital tract, breast, and placenta.**[26].** Although its primary purpose is to break down the HA-rich matrix around the egg to allow sperm cells to traverse, PH-20 is thought to be a multifunctional protein that may also be crucial for intercellular signaling **[27, 28].**

1.3.3. Hyaluronidases of Invertebrate

1.3.3.1. Hyase from Leech

Leech hyaluronidases (LHase), a member of the hyaluronate 3-glycanohydrolase family, cleave the β-1,3-glucuronide bonds to break down HA into (GlcNAcGlcUA)2 and (GlcNAc-GlcUA)3, effectively producing oligomers of glucuronic acid on the reducing end **[29].** Yuki and Fishman carried out the purification followed by characterization of hyaluronidase in 1963 **[30].**

1.3.3.2. Hookworm Hyaluronidases

In hookworms, hyaluronidase activity has also been found by researchers. According to Hotez et al. Hyaluronidase from Ancylostoma hookworm larvae is comparable to that of leech hyaluronidase in that it has a high HA selectivity and an optimum pH that is somewhat neutral. **[31].**

1.3.4. Microbial hyaluronidases

The majority of recognized hyaluronidase enzymes derived from microbes belong to the class of hyaluronate lyase, this type of enzyme uses β-elimination to break the 1,4

glycosidic bonds in HA, ultimately forming disaccharides but are unsaturated. Microorganisms have been reported to possess a wide distribution of hyaluronate lyases. Girish and Kemparaju compiled a list of microbes that produce hyaluronate lyase **[8],** and since then, additional microbial hyaluronidases have been found. Pathogenic bacteria are assumed to be infected through the action of hyaluronate lyases. Hyaluronate lyase acts as a spreading factor for bacterial invasion by breaking down the extracellular matrix (ECM) however, the breakdown product can also be utilized as a source of carbon for the development of bacteria **[32].**

1.3.4.1. Microorganisms producing hyaluronidase

Numerous distinct bacterial genera generate hyaluronidases **[33]**. By dissolving the components of the host's extracellular matrix, gram-positive bacteria's HA lyases are thought to operate as virulence factors, aiding the spread of diseases or poison **[34]**. Hyaluronidase-mediated degradation of HA results in increased tissue permeability which is thought to contribute to a range of medical conditions, such as wound infections, syphilis, gangrene, meningitis, synovitis, , pneumonia, and mastitis.. **[35, 36, 37].** Numerous species of bacteria such as *Streptococcus spp, Staphylococcus spp, Streptomyces spp,* and *Clostridium spp* are among the gram-positive microbes that may produce hyaluronidase **[38, 39 ,40, 41 , 42].**

Gram-negative bacteria do not secrete hyaluronidase into the extracellular matrix, hence they are less likely to contribute to disease **[34].**It was found that *S. pneumoniae* and *S. agalactiae* cultures contain hyaluronidase which implies that during infection, the pathogen releases some of the enzyme to adjacent host tissues to facilitate bacterial invasion. **[37, 43].**

1.4. Assays for hyaluronidases activity

The turbidity reduction assay was used to assess the hyaluronidase activity spectrophotometrically using HA sodium salt as a substrate by incubating 1 ml of HA at concentration of 70 g/ml with 1 ml of the enzyme sample in the presence of 0.05 M sodium phosphate buffer with 0.05 M NaCl (pH 7.0) at 37 °C for 30 min. After incubation, 2.5 ml of an acidified protein solution (1% w/v) of bovine serum albumin fraction (BSA) in 0.5 M sodium acetate buffer (pH 3.1) was added. The absorbance at 600 nm was measured to determine the degree of turbidity reduction. The unite of enzyme is the amount of enzyme that

induces a reduction in turbidity under specific conditions at 600 nm in 30 min. at 37°C, at pH 7.0, **[44].**

1.5. Production and purification approaches

The purification method is crucial to the many stages of industrial processing. Hyaluronidase is frequently purified employing a range of chromatographic methods, including gel filtration with medium columns like Sephadex G-100 chromatography, affinity chromatography, and ion exchange chromatography **[11].** Ion exchange chromatography uses an exam Q Sepharose fast flow column. With Indian cobra (Naja naja) venom, Sephadex G-75 and CM-Sephadex C-25 chromatography were utilized to purify Hyaluronidase. **[45].** Molecules are separated according to their size and shape using gel filtration chromatography. The larger molecules are more difficult to move through the pores of the adsorbent than smaller ones are. Due to the presence of extra proteins, this method of purification is not widely used for pharmacological applications **[46].** Guo et al. was isolated hyaluronidase from *Bacillus sp*. A50 strain using DEAE Sepharose Fast Flow column **[3].**

1.6. Physicochemical properties of hyaluronidases

Many species have been used to produce and characterize hyaluronidases. According to their pH, temperature and metal ions over the recent ten years. According to pH**,** hyaluronidases can be divided into two groups: acid-active hyaluronidases, which begin to function at a pH of 3 to 4, and neutral-active hyaluronidases, which begin to function at a pH of 5 to 8 **[5].** Temperature also is one of the most significant physical factors influencing the rate of hyaluronidase. It was noted that hyaluronidase from different sources has a wide range of temperatures for example, the hyaluronidase from *Bacillus sp*. A50 showed an ideal temperature of 44°C **[3]** while Patil et al **[47]** discovered that the enzyme's activity decreased significantly beyond 40 °C with 97% of its activity occurring in the 35–40 °C temperature range. Furthermore, it was found by El-Shanawany et al **[13]** that the reaction temperature with the greatest hyaluronidase activity by Streptomyces roseofulvus was recorded at 35°C .. Metal ions are also a very effective factor that influences hyaluronidase activity. It was reported by Guo et al **[3]** that Ca2, Mg2+, Ni2+, Co2+, and Ba2 are among the metal ions that positively impact the hyaluronidase activity that *Bacillus spp*. produces.

1.7. Applications and importance of hyaluronidase

1.7.1. Therapeutic uses of hyaluronidase

In numerous specialties, including ophthalmology, surgery, obstetrics, etc., hyaluronidase are frequently utilized therapeutically. In lens transplantation surgery, HA is frequently injected intraoperatively to preserve the anterior chamber of the eye or during lens transplantation to shield the corneal endothelium. Hyaluronidase injections are an effective way to prevent the concurrent increase in intraocular pressure **[48].** According to Harooni et al, hyaluronidase can be employed in posterior vitreous detachment **[49].**

1.7.2. Hyaluronidase as drug

Hyaluronidase is frequently used as a drug to increase tissue permeability by decreasing the viscosity of HA, a component of the extracellular matrix (ECM), through hydrolyzing it. As a result, it is used with other medications in medicine to accelerate their administration and dispersion. Ophthalmic surgery using local anesthetics is a common application. It also improves the pace at which parenteral fluids administered by hypodermoclysis are absorbed, and it is used as a supplement in subcutaneous urography to enhance the resorption of radiopaque substances. Extravasation of hyperosmolar solutions is another application for hyaluronidase. **[50].**

1.7.3. Role in fertilization

The outer layer of a typical mammalian egg is made up of roughly 3,000 cumulus cells that are embedded in an extracellular matrix that is high in HA **[18]**. Sperm hyaluronidase has been linked to sperm penetration of the cumulus oophorus' extracellular matrix and may be essential for gamete contact and mammalian fertility **[51]**. In the process of fertilization, Sperm and ovum come into contact in order to fuse and create a diploid zygote. During this procedure, sperm cell-anchored PH-20 enzyme collaborates with serine proteases of the sperm to help it to

pass through cumulus-oocyte complexes. **[52].** The hyaluronidase enzymes on the surface of a capacitated sperm help it break through this covering when it reaches the ovum. Following this, the sperm can adhere to the zona pellucida and initiate the acrosome response **[53].**

1.7.4. Role in filler injection

The most popular injectable dermal filler is HA but excessive quantities of the injected filler causes subcutaneous nodules **[54].** These subcutaneous nodules and excessive amounts of injectable filler can be removed using hyaluronidases **[5].** The amount of HA, the quantity of crosslinks, and the type of filler all affect how the filler reacts to hyaluronidase. Therefore, a sufficient dose of hyaluronidase must be administered close to a HA filler to breakdown it **(Fig 2)**. Hyaluronidase injection into the filler itself may be helpful if it is inserted subcutaneously; however, if the filler is inserted inside a blood vessel, it is sufficient to inject the enzyme around the vessel rather than into the filler itself. Hyaluronidase injection may produce allergic reactions. The majority of hyaluronidase allergic reactions are local, although rarely, systemic reactions can also happen **[2].**

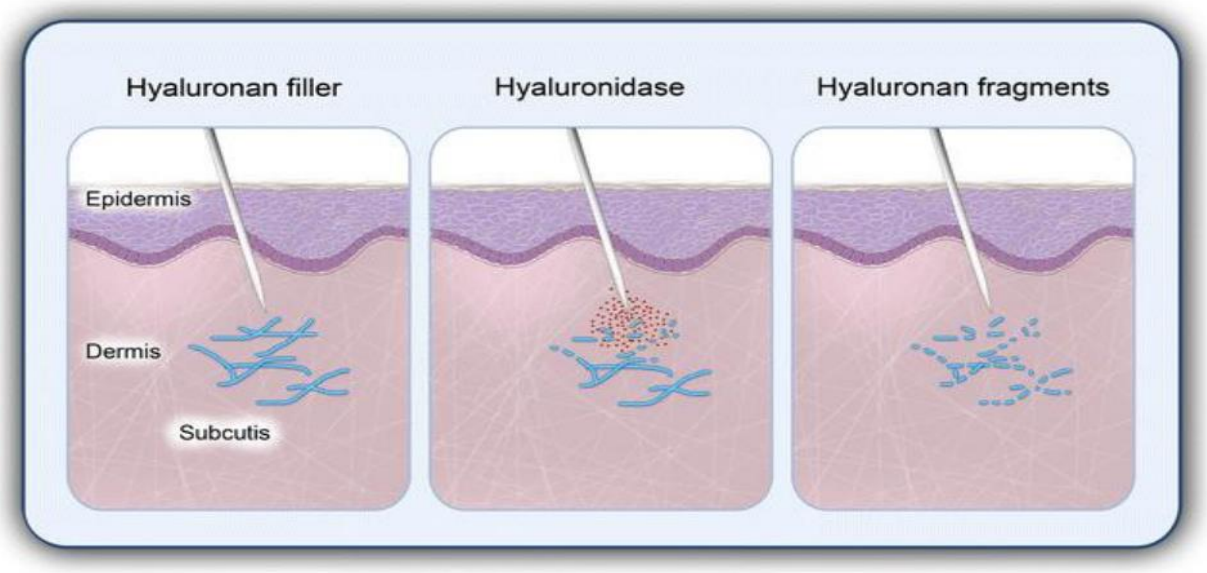

Fig. 2. Role of hyaluronidase enzyme in filler injection

1.7.5. Hyaluronidase for Cancer Therapy

Hyaluronidase degrades HA, which is mostly recognized to be present in the extracellular matrix of connective tissues **[55].** Testicular Hyaluronidase can be used to reverse acquired chemo-resistance caused by high levels of HA, a very hydrophilic substance, in the

tumor stroma. This increases interstitial pressure and prevents the infusion of chemotherapeutics. Because HA breakdown caused by Hyaluronidase activity can increase the cell membrane's absorptivity, Hyaluronidase is regarded as an efficient drug circulation agent. Hyaluronidase has the ability to enhance the passage of antibiotics towards the synovial fluid **[11].** Hyaluronidase alone is thought to be a very efficient counteragent to the extravasation of antineoplastic medicines like vinblastine, primarily in oncological therapy, since it can reduce tissue death and limit local necrosis by reducing extravasation absorption **[56] .** In short, hyaluronidase is very helpful for cancer treatment in two different methods **[57] .** The first method involves using hyaluronidase to break down extracellular matrix, which opens more space for chemotherapeutic drugs to enter tumors. The second method involves controlling the growth and progression of malignancies. Hyaluronidase has been included in chemotherapy for cancer to enhance penetration of drug. In clinical studies, testicular Hyaluronidase has been utilized to boost the effectiveness of vinblastine in the treatment of bladder cancer, glioma treated with boron neutron therapy, and malignant melanoma and Kaposi's sarcoma **[58].**

1.7.6. Hyaluronidase as antioxidant

Hyaluronidase enzyme included hydroxylic and carboxylic functional groups in its structure, which improved its antioxidant capabilities. It enhanced the antioxidant capacities of superoxide scavenging, total antioxidant, DPPH radical scavenging, and total reducing power **[59]**. Antioxidants are necessary to preserve the integrity of cells and consequently the host immune system's homeostasis. By preserving the redox reputation of the cells, the stability of pro-oxidant and antioxidant ranges controls the destiny of genomic integrity in cells **[60]**. It is widely known that free radicals, such as superoxide anion, hydroxyl radical, hydrogen peroxide, and nitric oxide are often referred to as ROS which can harm cells by interacting with plasma membranes' unsaturated fatty acids, as a result it reduces membrane fluidity and damages membrane proteins. For this reason, these antioxidants work to protect against cell damage and are crucial in adjuvant chemotherapy **[61]**. Antioxidants, which are found in natural therapies to treat Parkinson's disease, Alzheimer's disease, cardiovascular disease, cancer, and neurological disorders, prevent these effects **[62]**.

2. Conclusion

Hyaluronidase has been used in multiple applications such as medical, commercial and physiological. For this reason, great interest of this enzyme appears. Hyaluronidase enzyme has many types and can be extracted from different sources so, further researches are still essential, because hyaluronidases have different sources and possible variations in their mechanisms of a ction. Purification of hyaluronidase is also diverse due to different types of enzyme and different physiochemical properties of it. Briefly, we must shed light on this enzyme and many researches should be designed to know more about this interesting enzyme.

3. Conflicts Of Interest

The authors declare no conflict of interest.

References

[1] S. Patil and B. Chaudhari, "A simple, rapid and sensitive plate assay for detection of microbial hyaluronidase activity, " J. Basic Microbiol,57: (2017), https://doi: 10.1002/jobm.201600579, 358–36.

[2] H. Jung, "Hyaluronidase : An overview of its properties , applications , and side effects," Archives of Plastic Surgery, *47*(4): (2020), https://doi.org/10.5999/aps.00752, 297–300.

[3] X. Guo, Y. Shi, J. Sheng, and F. Wang, "A Novel Hyaluronidase Produced by Bacillus sp . A50, " journal.pone 9: (2014), https://doi: 10.1371/.0094156,1-8.

[4] N. S. El-Safory, A. E. Fazary, and C. K. Lee, "Hyaluronidases, a group of glycosidases: Current and future perspectives, " Carbohydrate Polymers 81:(2010), https://doi: 10.1016/j.carbpol.2010.02.047, 165-181.

[5] M. Cavallini, R. Gazzola, M. Metalla, and L. Vaienti, "The role of hyaluronidase in the treatment of complications from hyaluronic acid dermal fillers, " Aesthetic Surgery Journal 33: (2013), https://doi: 10.1177/1090820X13511970, 1168-1174.

[6] N. Kohno, T. Ohnuma, and P. Truog, "Effects of hyaluronidase on doxorubicin penetration into squamous carcinoma multicellular tumor spheroids and its cell lethality, " J. Cancer Res. Clin. Oncol 120: (1994), https://doi: 10.1007/BF01236386.

[7] M. Evison, C. Pretty, E. Taylor, C. Franklin, and V. Wing, "Human recombinant hyaluronidase (Cumulase®) improves intracytoplasmic sperm injection survival and fertilization rates, " Reprod. Biomed. Online 18: (2009), https://doi:10.1016/S1472-6483(10)60030-2, 811-814.

[8] K. S. Girish and K. Kemparaju, "The magic glue hyaluronan and its eraser hyaluronidase: A biological overview, " Life Sciences 80: (2007), https://doi: 10.1016/j.lfs.2007.02.037.

[9] G. B. Racz, J. E. Heavner, and A. Trescot, "Percutaneous lysis of epidural adhesions - Evidence for safety and efficacy, " Pain Practice 8: (2008), https://doi: 10.1111/j.1533-2500.2008.00203.x.

[10] A. L. Dunn, J. E. Heavner, G. Racz, and M. Day, "Hyaluronidase: A review of approved formulations, indications and off-label use in chronic pain management, " Expert Opinion on Biological Therapy10: (2010), https://doi: 10.1517/14712590903490382.

[11] A. Shakouri, R. Parvan, N. Adljouy, and J. Abdolalizadeh, "Purification of hyaluronidase as an anticancer agent inhibiting CD44, " Biomedical Chromatography 34: (2020), https://doi: 10.1002/bmc.4709, 1-9.

[12] A. Hamai, K. Mordcawa, and K. Horie, "Purification and Characterization of Hyaluronidase from Streptococcus dysgalactiae, " Agric. Biol. Chem 53: (1989), https://doi.org/10.1016/S0934-8840(11)80509-8, 2163–2168.

[13] F. M. Reda and S. M. El-Shanawany, "Characterization and immobilization of a novel hyaluronidase produced by Streptomyces roseofulvus, " Egypt. J. Bot 60: (2020), https://doi: 10.21608/ejbo.2019.6242.1248, 213–224.

[14] L. Wang *et al.*, "Characterization of a Hyaluronidase - Producing Bacillus sp . CQMU - D Isolated from Soil, " Current Microbiology 79(11) : (2022), https://doi: 10.1007/s00284-022-03035-9, 1–9.

[15] A. B. Csoka, G. I. Frost, and R. Stern, "The six hyaluronidase-like genes in the human and mouse genomes, " Matrix Biology 20: (2001), https://doi: 10.1016/S0945-053X(01)00172-X, 499–508.

[16] G. Lepperdinger, J. Müllegger, and G. Kreil, "Hyal2 - Less active, but more versatile?, " Matrix Biology 20: (2001), https://doi: 10.1016/S0945-053X(01)00170-6, 509-514.

[17] D. Jiang, J. Liang, and P. W. Noble, "Hyaluronan in tissue injury and repair, " Annual Review of Cell and Developmental Biology 23: (2007), https://doi: 10.1146/annurev.cellbio.23.090506.123337, 435–461.

[18] Y. Lin, K. Mahan, W. F. Lathrop, D. G. Myles, and P. Primakoff, "A hyaluronidase activity of the sperm plasma membrane protein PH-20 enables sperm to penetrate the cumulus cell layer surrounding the egg, " J. Cell Biol., 125: (1994), https://doi: 10.1083/jcb.125.5.1157, 1157–1163.

[19] K. Weissmann & Meyer, "'Hyaluronidases, '" Acad. Press, (1971), New York.

[20] A. C. Terwisscha van Scheltinga, S. Armand, K. H. Kalk, A. Isogai, B. Henrissat, and B. W. Dijkstra, "Stereochemistry of Chitin Hydrolysis by a Plant Chitinase/Lysozyme and

X-ray Structure of a Complex with Allosamidin: Evidence for Substrate Assisted Catalysis," Biochemistry 34: (1995), https://doi: 10.1021/bi00048a003, 15619–15623.

[21] T. Barth *et al.*, "Biochemical and structural characterization of a protein complex containing a hyaluronidase and a CRISP-like protein isolated from the venom of the spider Acanthoscurria natalensis, " J. Proteomics 192: (2019), https://doi: 10.1016/j.jprot.2018.08.012, 102-113.

[22] J. Frangieh *et al.*, "First characterization of the venom from apis mellifera syriaca, a honeybee from the middle east region, " Toxins (Basel) 11: (2019), https://doi: 10.3390/toxins11040191.

[23] K. Kemparaju and K. S. Girish, "Snake venom hyaluronidase: A therapeutic target, " Cell Biochemistry and Function 24: (2006), https://doi: 10.1002/cbf.1261, 7-12.

[24] M. Gmachl and G. Kreil, "Bee venom hyaluronidase is homologous to a membrane protein of mammalian sperm, " Proc. Natl. Acad. Sci. U. S. A 90: (1993), https://doi: 10.1073/pnas.90.8.3569, 3569–3573.

[25] Z. Marković-Housley, G. Miglierini, L. Soldatova, P. J. Rizkallah, U. Müller, and T. Schirmer, "Crystal structure of hyaluronidase, a major allergen of bee venom," Structure 8: (2000), https://doi: 10.1016/S0969-2126(00)00511-6.

[26] A. B. Csóka, S. W. Scherer, and R. Stern, "Expression analysis of six paralogous human hyaluronidase genes clustered on chromosomes 3p21 and 7q31, " Genomics 60: (1999), https://doi: 10.1006/geno.1999.5876, 356-361.

[27] G. N. Cherr, A. I. Yudin, and J. W. Overstreet, "The dual functions of GPI-anchored PH-20: Hyaluronidase and intracellular signaling, " Matrix Biology, 20: (2001), https://doi: 10.1016/S0945-053X(01)00171-8, 515–525.

[28] C. A. Vines, M. W. Li, X. Deng, A. I. Yudin, G. N. Cherr, and J. W. Overstreet, "Identification of a hyaluronic acid (HA) binding domain in the PH-20 protein that may function in cell signaling, " Mol. Reprod. Dev 60: (2001), https://doi: 10.1002/mrd.1119, 542–552.

[29] A. Linker, P. Hoffman, and K. Meyer, "The hyaluronidase of the leech: An endoglucuronidase [11], " Nature 180: (1957), https://doi: 10.1038/180810b0, 810–811.

[30] H. Yuki and W. H. Fishman, "Purification and characterization of leech hyaluronic acid-endo-beta-glucuronidase., " J. Biol. Chem 238: (1963), https://doi: 10.1016/s0021-9258(18)81151-6, 1877–1879.

[31] P. J. Hotez *et al.*, "Hyaluronidase from infective Ancylostoma hookworm larvae and its possible function as a virulence factor in tissue invasion and in cutaneous larva migrans, " Infect. Immun 60: (1992), https://doi: 10.1128/iai.60.3.1018-1023.1992, 1018–1023.

[32] R. Stern and M. J. Jedrzejas, "Hyaluronidases: Their genomics, structures, and mechanisms of action, " Chemical Reviews 106: (2006), https://doi: 10.1021/cr050247k, 818-839.

[33] M. J. Jedrzejas, "Pneumococcal Virulence Factors: Structure and Function, " Microbiol. Mol. Biol. Rev 65: (2001), https://doi: 10.1128/mmbr.65.2.187-207.2001.

[34] W. L. Hynes and S. L. Walton, "Hyaluronidases of Gram-positive bacteria, " FEMS Microbiology Letters 183: (2000), https://doi: 10.1016/S0378-1097(99)00669-2, 201-207.

[35] O. Matsushita and A. Okabe, "Clostridial hydrolytic enzymes degrading extracellular components, " Toxicon 39: (2001), https://doi: 10.1016/S0041-0101(01)00163-5, 1769-1780.

[36] G. Makris, J. D. Wright, E. Ingham, and K. T. Holland, "The hyaluronate lyase of Staphylococcus aureus - A virulence factor?, " Microbiology 150: (2004), https://doi: 10.1099/mic.0.26942-0, 2005-2013.

[37] S. Li, S. J. Kelly, E. Lamani, M. Ferraroni, and M. J. Jedrzejas, "Structural basis of hyaluronan degradation by Streptococcus pneumoniae hyaluronate lyase, " EMBO J 19: (2000), https://doi: 10.1093/emboj/19.6.1228, 1228-1240.

[38] E. Günther, J. H. Ozegowski, and W. Köhler, "Occurrence of extracellular hyaluronic acid and hyaluronatlyase in streptococci of groups A, B, C, and G, " Zentralblatt fur Bakteriol 285: (1996), https://doi: 10.1016/S0934-8840(96)80023-5, 64-73.

[39] A. M. Berry, R. A. Lock, S. M. Thomas, D. P. Rajan, D. Hansman, and J. C. Paton, "Cloning and nucleotide sequence of the Streptococcus pneumoniae hyaluronidase gene and purification of the enzyme from recombinant Escherichia coli, " Infect. Immun 62: (1994), https://doi: 10.1128/iai.62.3.1101-1108.1994, 1101-1108.

[40] T. J. Fitzgerald and E. M. Gannon, "Further evidence for hyaluronidase activity of Treponema pallidum, " Can. J. Microbiol 29: (1983), https://doi: 10.1139/m83-232, 1507-1513.

[41] B. Canard, T. Garnier, B. Saint-Joanis, and S. T. Cole, "Molecular genetic analysis of the nagH gene encoding a hyaluronidase of Clostridium perfringens, " MGG Mol. Gen. Genet 243: (1994), https://doi: 10.1007/BF00280319, 215-224.

[42] K. Girish, K. Kemparaju, S. Nagaraju, and B. Vishwanath, "Hyaluronidase Inhibitors: A Biological and Therapeutic Perspective, Curr. Med. Chem 16, : (2009), https://doi: 10.2174/092986709788453078, 2261-2288.

[43] M. J. Jedrzejas and L. Chantalat, "Structural studies of Streptococcus agalactiae hyaluronate lyase, " Acta Crystallogr. Sect. D Biol. Crystallogr 56, : (2000), https://doi: 10.1107/S0907444900000706, 460–463.

[44] B. Y. Albert and L. Ott, "A Turbidimetric method for the assay of hyaluronidase a variety of methods have been devisedfor the assay of the enzyme These may be divided into four types : (a) measurement of the ' spreading effect ' in the skin of rabbits and guinea p, " J. Biol. Chem 172: (1947), https://doi: 10.1016/S0021-9258(19)52721-1, 367–375.

[45] K. S. Girish, R. Shashidharamurthy, S. Nagaraju, T. V. Gowda, and K. Kemparaju, "Isolation and characterization of hyaluronidase a 'spreading factor' from Indian cobra (Naja naja) venom, " Biochimie 86: (2004), https://doi: 10.1016/j.biochi.2004.02.004.

[46] A. Kuhn, "Correspondence regarding Zhong et al., BMC Bioinformatics 2013 Mar 7;14:89," *BMC Bioinformatics*, vol. 15, no. 1, pp. 2–4, 2014, doi: 10.1186/s12859-014-0347-5.

[47] S. P. Patil, L. P. Shirsath, and B. L. Chaudhari, "A halotolerant hyaluronidase from newly isolated Brevibacterium halotolerans DC1: Purification and characterization, " Int. J. Biol. Macromol 166: (2021), https://doi: 10.1016/j.ijbiomac.2020.10.240, 839–850.

[48] W. C. Hubbard *et al.*, "Intraocular pressure and outflow facility are unchanged following acute and chronic intracameral chondroitinase ABC and hyaluronidase in monkeys, " Exp. Eye Res 65: (1997), https://doi: 10.1006/exer.1997.0319, 177–190.

[49] M. Harooni, T. McMillan, and M. Refojo, "Efficacy and safety of enzymatic posterior vitreous detachment by intravitreal injection of hyaluronidase, " Retina 18: (1998), https://doi: 10.1097/00006982-199818010-00004, 16-22.

[50] J. C. Houck and C. M. Chang, "Permeability factor contaminating hyaluronidase preparations, " Inflammation 3: (1979), https://doi: 10.1007/BF00913502, 447-451.

[51] S. A. Meyers and A. E. Rosenberger, "A plasma membrane-associated hyaluronidase is localized to the posterior acrosomal region of stallion sperm and is associated with spermatozoal function, " Biol. Reprod 61: (1999), https://doi: 10.1095/biolreprod61.2.444, 444-451.

[52] N. Kawano *et al.*, "Mice lacking two sperm serine proteases, ACR and PRSS21, are subfertile, but the mutant sperm are infertile in vitro, " Biol. Reprod 83: (2010), https://doi: 10.1095/biolreprod.109.083089, 359-369.

[53] Y. Zhang, J. Gong, Z. Yao, J. Jiang, C. Su, and H. Li, "Insights into the source , mechanism and biotechnological applications of hyaluronidases, " Biotechnol. Adv 60: (2022), https://doi: 10.1016/j.biotechadv.2022.108018, 1-18.

[54] S. S. Kwak *et al.*, "Comparative analysis of hyaluronidase-mediated degradation among seven hyaluronic acid fillers in hairless mice, " Clin. Cosmet. Investig. Dermatol 14: (2021), https://doi: 10.2147/CCID.S300960, 241–248.

[55] A. F. Wahby *et al.*, "Role of hyaluronidase inhibitors in the neutralization of toxicity of Egyptian horned viper Cerastes cerastes venom, " J. Genet. Eng. Biotechnol 10,: (2012), https://doi: 10.1016/j.jgeb.2012.10.001, 213–219.

[56] X. Yao *et al.*, "Guidelines for Diagnosis and Treatment of Atopic Dermatitis in China, " Int. J. Dermatology Venereol 4: (2021), https://doi: 10.1097/JD9.0000000000000143, 1-9.

[57] V. B. Lokeshwar, W. H. Cerwinka, T. Isoyama, and B. L. Lokeshwar, "HYAL1 hyaluronidase in prostate cancer: A tumor promoter and suppressor, " Cancer Res 65: (2005), https://doi: 10.1158/0008-5472.CAN-05-1022, 7782-9.

[58] C. O. McAtee, J. J. Barycki, and M. A. Simpson, "Emerging roles for hyaluronidase in cancer metastasis and therapy, " in Advances in Cancer Research 123: (2014), https://doi: 10.1016/B978-0-12-800092-2.00001-0, 1-34.

[59] K. Thirumurthy, K. Kaliyamoorthy, and K. Kandasamy, "Antioxidant and Anti-Breast Cancer Properties of Hyaluronidase from Marine Staphylococcus aureus (CASMTK1), " J. Marine. Sci. Eng 11: (2023), https://doi.org/10.3390/jmse11040778, 1-21.

[60] P. Anitha, J. Bhargavi, G. Sravani, B. Aruna, and S. Ramkanth, "Recent progress of dendrimers in drug delivery for cancer therapy, " International Journal of Applied Pharmaceutics 10: (2018). https://doi: 10.22159/ijap.2018v10i5.27075.

[61] V. Afonso, R. Champy, D. Mitrovic, P. Collin, and A. Lomri, "Reactive oxygen species and superoxide dismutases: Role in joint diseases, " Joint Bone Spine 74: (2007), https://doi: 10.1016/j.jbspin.2007.02.002, 324-329.

[62] M. Valko, D. Leibfritz, J. Moncol, M. T. D. Cronin, M. Mazur, and J. Telser, "Free radicals and antioxidants in normal physiological functions and human disease, " International Journal of Biochemistry and Cell Biology 39: (2007), https://doi: 10.1016/j.biocel.2006.07.001, 44-84.

الملخص العربي

نظرة عامة على أنواع إنزيم الهيالورونيديز وطريقة عمله وقياسه وتطبيقاته العلاجية.

مي عبد النبي إبراهيم [١]*، إسماعيل محمد الفخراني [٢،٣]، شريف موسى حسيني [١]، فافي عبد الرحمن محمد [١].

[١] قسم النبات، كلية البنات للآداب والعلوم والتربية، جامعة عين شمس، مصر.
[٢] قسم بحوث البروتين، معهد بحوث الهندسة الوراثية والتكنولوجيا الحيويةGEPRI ، مدينة البحث العلمي والتطبيقات التكنولوجية(SRTA-City) ، برج العرب الجديدة ٢١٩٣٤، الإسكندرية، مصر.
[٣] مركز تطوير الصناعات الدوائية والتخمير(PFIDC) ، مدينة الأبحاث العلمية والتطبيقات التكنولوجية(SRTA-City) ، برج العرب الجديدة ٢١٩٣٤، الإسكندرية، مصر.

الملخص العربي

يعتبر إنزيم الهيالورونيديز هو إنزيم العصر الحالي, ليس فقط لأهميته الطبية في علاج السرطان ولكن أيضًا لأهميته الكبيرة في العديد من المجالات. تتمتع مجموعة انزيمات الهيالورونيديز بنشاط بيولوجي كبير في مجال الطب بالإضافة إلى كونها إنزيمات مفيدة لإنتاج حمض الهيالورونيك منخفض الوزن الجزيئي. يتم استخدامه علاجيا كمحفز مهم لنشر الدواء للمساعدة في امتصاص الدواء والورم الدموي الموضعي أو تبديد الوذمة بعد الصدمة أو الجراحة. إن إنزيم الهيالورونيداز المستخلص من الحيوانات له بعض العيوب، بما في ذلك قلة الموارد، والمحتوى الضعيف، والتكلفة العالية، وإمكانية الإصابة بالفيروسات. على النقيض من ذلك، يتم استخدام الهيالورونيداز المستخلص من الكائنات الحية الدقيقة على نطاق واسع بسبب مصدره الوفير، وإجراءات التخمير المباشرة، والتكلفة المنخفضة، والنقاء العالي، والحد الأدنى من التلوث البيئي. تُعرف إنزيمات الهيالورونيداز الميكروبية باسم hyaluronate lyases نظرًا للروابط المزدوجة غير المشبعة التي تتشكل عند الأطراف غير المختزلة عن طريق تكسير حمض الهيالورونيك من خلال تفاعل القضاء على بيتا. تم العثور على العيد من الميكروبات التي تحتوي على انزيم الهيالورونيديز مثل البكتريا الكرويه وكذلك العنقوديه.