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Review

Hyaluronidases, a group of glycosidases: Current and future perspectives

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ABSTRACT

Given the constant synthesis and degradation of hyaluronan (hyaluronic acid, HA) in tissues, it is remarkable that the human body maintains precise levels of hyaluronan as tightly as it does. Hyaluronidases represent a group of glycosidases, which mainly degrade hyaluronan, a linear, non-sulfated polysaccharide composed of repeating disaccharide units [D-glucuronic acid (1-β-3) N-acetyl-D-glucosamine (1-β-4)]n. They are widely distributed in nature, being found in mammals, insects, leeches and bacteria. There has been a plethora of review articles on hyaluronidases, either as a subject on their own or as part of a review on glycosidases enzymes. The present review summarizes the current research on their classification, sources, activity assays, bio-physical and chemical properties, crystal structural features and its catalytic mechanism. Special emphasis is given to the importance role of that type of enzymes in biotechnological processes, as well as its medicinal and bio-industrial applications.

Contents

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1. Introduction

Hyaluronidases (Hyals) are classes of glycosidases that predominately degrade hyaluronic acid (HA), with limited ability to degrade chondroitin and chondroitin sulphates (Table 1). Also, hyaluronidases are endoglycosidases, as they can degrade the β-N-acetyl-D-glucosaminidic linkages in the HA polymer. Since it was known in the first half of the last century, hyaluronidases have become with considerable roles in biotechnological processes mainly in pharmaceutical industry. Therefore, the discovery of new hyaluronidases with novel properties continues to be an important research area. The amount of research work directed towards hyaluronidases has increased dramatically since 1940. For example, over the periods 1940–1960 and 1960–present, the average number of refereed publications describing research involving hyaluronidases as a major component (within abstract, title, and keywords; using Scopus search engine) was 420 and 8004, respectively. In the last decade (2000–2009), it is interesting to note that the publications concerned with hyaluronidases observed between 2000 (201 documents) and 2009 (239 documents) coincides with the most recent discoveries on isolation, purification, characterization and applications of hyaluronidases. There were also striking increases in the number of publications concerned with hyaluronic acid (the related compound to hyaluronidases). The average number of documents was 92 for the period (1940–1960), while for the period 1960–present, the average number was 21684 using the same search engine (Scopus). Among all of this research work, a very few reports were found in literature describe concisely hyaluronidases types. In this review, we concentrate on the previous relevant studies conducted in hyaluronidases sources, their production, bio-physico-chemical properties, crystal structural features, and finally their biological functions.

Hyaluronan (hyaluronic acid, or hyaluronate, HA) (Fig. 1) is a high-molecular mass polysaccharide found in the extracellular matrix, especially of soft connective tissues. Commonly, hyaluronan is known as a lubricant responsible for the viscoelastic properties of tissue fluids and as a stabilizing and hydrating component of soft connective tissue. The chemical structure of hyaluronic acid of repeating disaccharide units linked by β-1,4 glycosidic bonds has been elucidated in which each disaccharide unit consists of D-glucuronic acid (GlcUAc) and N-acetyl-D-glucosamine (GlcNAc) connected by a β-1,3 glycosidic bond. Depending on the tissue source, this polymer usually consists of 2000–25000 disaccharides, giving rise to molecular masses ranging from 10^6 to 10^7 Da with extended lengths of 2–25 μm. Under *in vivo* conditions hyaluronic acid exists as a polyanion as the carboxyl groups of the glucuronic acid ause the high negative charge of the polymer under physiological conditions. In other words, hyaluronic acid is a high-molecular weight, highly anionic glycosaminoglycans (GAG) (Fig. 1), including chondroitin-, dermatan- and keratan sulphate, heparin and heparan sulphate.

The described secondary structure of HA allows for a further organization of the macromolecule in an aqueous environment and its tertiary structures are specifically and reversibly disaggregated by mild physicochemical methods (Scott, Cummings, Brass, & Chen, 1991; Scott & Heatley, 2002). This dynamic meshwork provides the basis for the viscoelastic and hydrating properties of hyaluronan. In solutions, between the HA chains a huge amount of water can be trapped, which can be withdrawn upon application of an external pressure causing the resilience and malleability of substances like synovial joint fluids (Toole, 2004; Prehm, 1984, 1990, 2006). In addition, the hydrophobic interactions and hydrogen bonds that can form between HA chains are counteracted by electrostatic repulsion of the carboxyl groups (Scott, Heatley, & Hull, 1984; Heatley & Scott, 1988; Heldin & Pertoft, 1993). Thus, HA is able to form electrostatic complexes with many proteins, including hyaluronidases (Vincent & Lenormand, 2009). Under the physiological conditions, the supramolecular organization of HA was found to be on the edge of stability, suggesting that reversible formation and breakdown of tertiary structures control important biological properties (Toole, 2004; Scott & Heatley, 1999).

Hyaluronan is found in the extracellular matrix of all vertebrates and in the capsule of some bacteria (Stern, 2004; Prehm, 1984, 1990, 2006; He et al., 2009). There are three reports (Laurent & Fraser, 1992; Necas, Brauner, & Kolar, 2008; Stern, 2004) describe metabolisms, physiological and pathological functions, basic pharmacological properties, and the clinical use of hyaluronic acid. The functions of hyaluronan directly originating from its physicochemical properties are well-known: it acts as a lubricant and shock absorber, regulates water balance and osmotic pressure and occurs as a structure forming molecule in the vitreous humor of the eye, in Wharton's jelly and in joint fluids. Hyaluronan fulfils several distinct physiological functions that contribute both to structural properties of tissues and to cell behaviour during formation or remodelling of tissues (Knudson, Bartnik, & Knudson, 1993). In addition to the functions arising directly from the physicochemical properties of the polymer, hyaluronan also exerts biological effects via specific interactions with hyaluronan-binding proteins (hyaladherins) (El Maradny et al., 1997; Evanko & Wight, 1999). The great number of hyaladherins known so far can be grouped into (i) the structural hyaluronan-binding proteins of the extracellular matrix, such as link protein and the aggregating proteoglycans, (ii) cell surface hyaluronan receptors and (iii) intracellular hyaluronan-binding proteins (Feinberg & Beebe, 1983; Prehm, 2002).

2. Classification of hyaluronidases

Molecular genetic analysis has shown that, hyaluronidases can be grouped according to amino acid sequence homology (Csoka, Frost, Wong, & Stern, 1997; Csoka, Frost, & Stern, 1997; Csoka, Frost, & Stern, 2001) into two main families: the hyaluronidases from eukaryotes and from prokaryotes. The first classification of hyaluronidases was established according to their catalytic mechanism into three main families (Meyer, 1971). This classification was based on substrate specificity and on biochemical analysis of the hyaluronidases and their reaction products (Fig. 2).

The first group of hyaluronidases are the hyaluronate 4-glycanohydrolases, mammalian hyaluronidases (EC 3.2.1.35) which degrading HA by cleavage of the β-1,4-glycosidic bond furnishing tetrasaccharide molecule as the main product. These enzymes are glycosidases with both hydrolytic and transglycosidase activity and degrade HA, chondroitin, chondroitin-4, 6-sulphate to a small extent, dermatan sulphate. The best known enzymes of this class are testicular, lysosomal and bee venom hyaluronidase.