BIOTRANS
LA ROCHELLE 2023

N°464 / PC
TOPIC(s) : Enzyme discovery and engineering / Enzyme production, immobilization

New hyaluronic acid degrading enzymes from fungi

AUTHORS

MARINA MINGUET-LOBATO / CATALYSIS INSTITUTE CSIC, C/ MARIE CURIE 2, MADRID
FADIA V. CERVANTES DOMINGUEZ / CATALYSIS INSTITUTE CSIC, C/ MARIE CURIE 2, MADRID
DAVID FERNANDEZ-POLO / CATALYSIS INSTITUTE, C/ MARIE CURIE 2, MADRID
FRANCISCO J. PLOU / CATALYSIS INSTITUTE, MADRID, MADRID
MARIA FERNANDEZ-LOBATO / MOLECULAR BIOLOGY SEVERO OCHOA CENTER UAM-CSIC, C/ NICOLAS CABRERA 1, MADRID

PURPOSE OF THE ABSTRACT

Hyaluronic acid or hyaluronan (HA) is a linear anionic non-ramified and non-sulphated glycosaminoglycan (GAG) composed by repeat units of β-1,4-D-glucuronic acid-β-1,3-N-acetyl-D-glucosamine (Fig. 1). HA plays numerous roles in biological processes due to its unique structural properties, as mechanical support of cells being the major component of extracellular matrix, virulence and cell signalling, migration and proliferation. Hyaluronidases (HAases) comprise a group of enzymes, widely distributed in all kingdoms of life, that primordially degrades HA, although possess activity towards other GAGs (chondroitin and its derivatives), thus participating in the referred processes as well. These enzymes can be divided in three types according to their enzymatic activities: (1) HA endo-β-1,4-acetyl-hexosaminidases, which cleave β-1,4 glycosidic linkages to form oligomers with tetrasaccharides as final products and with GlcNAc at the reducing end (mainly found in mammals); (2) HA endo-β-1,3-glucuronidases, which cleave β-1,3 glycosidic linkages yielding tetra- or hexa-saccharides with a GlcNAc at the non-reducing end and GlcA at the reducing end (found in leeches); (3) HA lyases, which catalyse β-elimination reactions yielding unsaturated carbon-carbon bond products, with unsaturated disaccharides as final products (mainly found in bacteria). In recent years, these enzymes have aroused enormous interest in the scientific community due to the increasing number of biotechnological applications as drug delivery, cosmetic surgeries, cancer therapies, HA processing and hyalo-oligosaccharides (HAOS) preparation [1, 2]. Among microbial sources, fungi hyaluronidases are less studied in comparison with bacterial ones. The aim of this work was to find new microbial sources of hyaluronidases focusing on that coming from fungi and the characterisation of their ability to degrade HA.

A mycelial microorganism not yet characterized was isolated from colloidal chitin containing minimal media and was screened for the discovery of enzymatic degrading activities towards different polysaccharides (basically chitin and HA) with positive result. This positive HA-degrading, apparently fungus, was cultivated in different developed screening media for HAase activity. Extracellular HAase expression was achieved to its maximum in a minimal medium containing HA as carbon source. The optimal pH and temperature of crude enzymatic extract for the degradation of high molecular weight HA were set at 4.5 and 50 °C. Time-course HA degradation was monitored by HPAEC-PAD and SEC-ELSD developed methods from cultures and reactions with the protein extracts. Maximal activity was achieved after 48 h of expression, although activity was observed after only 6 h of expression on cultures. Main HAOS formed upon prolonged times of reaction were characterized by MALDI-TOF MS. Product analysis confirmed that main positive mode detected oligomers contained an unsaturation and, therefore, were produced in a β-elimination manner. However, oligosaccharides without unsaturation were also detected in negative mode, being the minimal polymerization degree a tetrasaccharide, thus suggesting the presence of an HA-4-glycanohydrolase enzyme. To our knowledge, this is the first time that two different HA degradation modes

were detected from an unique fungal organism. Our results suggest a multi-enzymatic attack to high molecular weight degradation and we are currently performing transcriptomic analysis of this microorganism in order to characterise the responsible genes of the observed enzymatic activities.

FIGURES

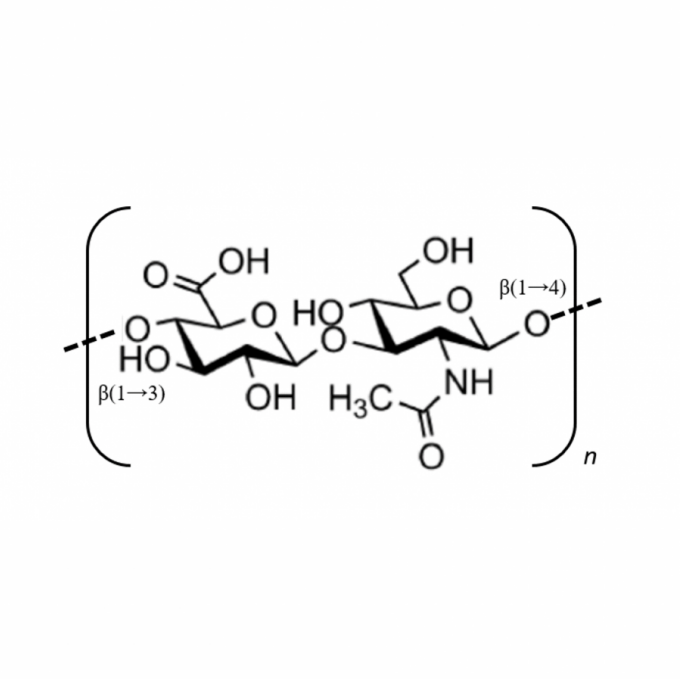

FIGURE 1

Hyaluronic acid chemical structure representation.

Structural unit of hyaluronan polymer consisted of N-acetyl-D-glucosamine and D-glucuronic acid. Glycosydic linkage types between sugar units are indicated.

FIGURE 2

KEYWORDS

hyaluronic acid | lyases | glycosidases | hyaluronidases

BIBLIOGRAPHY

[1] yue-sheng z., biotch. adv. 2022, 60, 108018.

[1] sliadovskii d., sci. rep. 2021, 11, 22600.