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The enzymology of *Streptococcus pyogenes* hyaluronidase

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ABSTRACT

"Necrotizing fasciitis, Glomerular-Nephritis, Pharyngitis (Strept Throat), Impetigo" the genesis of all of these diseases is due to the jeopardous microbe "*Streptococcus pyogenes*". The capsular Hyaluronic Acid (HA) in *Streptococcus pyogenes* serves as a camouflage, baffling the host immune response. *S.pyogenes* accesses the host cell through the enzyme "Hyaluronidase" which degrades the Hyaluronic acid in the Extracellular matrix of the host cells. Hyaluronidase- analogous to a nozzle speeds up the bacterial penetration in and around the host cell, thereby daggering the life of host cells. Curbing the anabolism or catabolism of HA by demodulating the enzymes would help in knocking out the infection. *Streptococcus pyogenes* can be penalized for its offense by whipping it again and again with a potent weapon (drug).

KEYWORDS

Streptococcus pyogenes;
Hyaluronic acid;
Hyaluronidase;
Extracellular matrix;
Pharyngitis.

HOW DOES THE "FLESH-EATING" BACTERIA ACCESS THE VIRTUOUS HOST?- AN INTRODUCTION

The accomplice here is the enzyme "Hyaluronidase" (HyaluronateLyase). Extracellular milieu of the human cells contains a polysaccharide named Hyaluronic acid (HA) a polymer with the monomers being N-Acetylglucosamine (NAG) (β1'!3) and D-Glucuronic acid (β1'!4)[1,2] This HA is present in vertebrates and capsule of *Streptococcus pyogenes* (Botzki A, 2004). HA synthase (encoded by Has operon) is the cracker-jack in production of HA and the produced HA is exported out the cell via an ABC transporter system[3,4]. HA in the capsule thwarts the attachment of the bacterial cells to the host macrophages which helps the bacteria in sidestepping the immune response. HA serves as the pabulum of Hyaluronidase. This enzyme degrades the HA and promotes the invasion of the virtuous host cells by the deadly bacteria.

WHY DOES HYALURONIDASE DEPOLYMERIZE HA?

The host connective tissues contain the viscous HA which provides a resistance force to the bacterial mo-

tion in the vicinity as a result of which the permeability of toxins & bacterial access to the connective tissues are restricted. In the presence of an activated hyaluronidase in the bacteria, the resistance offering component (HA) is wiped off & the host cell indirectly is made to welcome the bacteria in its neighbourhood. In the latter case the bacterial access and the permeability of toxins into the host cells is favored facilitating the bacterial growth.

This is the lions' share of hyaluronidase during an infection. Any infection related to this bacterium can be subsided in 2 ways: (a) Muffling hyaluronate synthase activity (leads to elimination of capsular HA) which will trigger the host immune response directly but this has no effect on *Streptococcus pyogenes* entry into the epithelial cells[5]. (b) Developing a novel and potent inhibitor against the bacterial assistant "hyaluronidase".

CAN STREPTOCOCCUS PYOGENES GOBBLE UP ITS OWN CAPSULAR HA?

It is well established that Hyaluronidase without the hyaluronic acid will be like a fish out of water. It will be completely inactive in this state on the contrary it would be extremely active and agile in the presence of its substrate Hyaluronic acid. It is an established fact that a structural synonymity exists between the HA of the mammalian cells to that of the HA of the Group A *Streptococci*.

If this is the situation: (A) Whether bacterial hyaluronidase can degrade its own capsular HA under normal conditions? (B) Is that the genes for hyaluronidase are substantially regulated??? Such that it doesn't degrade its own HA under normal conditions or (C) Is there an existence of a factor external to the bacterial cell that prevents this kind of degradation? Apart from these there is one more possibility that there might be an incessant manufacturing of the enzyme during the infection which may depolymerize the capsular HA along with the host cell HA. Still in this case the un-encapsulated bacteria might be protected from the host phagocytic machinery due to the possession of virulence factors[6]. These questions conflict with the minds while thinking about bacterial hyaluronidase however they need to be unlocked to this world through future research in this enzyme.

IS STREPTOCOCCUS PYOGENES – CARRIER OF TWINS?

Not every strain of *Streptococcus pyogenes* produces detectable hyaluronidase. It is the property of the strain & the serotype to produce ample or meager quantities of the enzyme. Certain bacteriophage contains genes that encode for hyaluronidase which missions to destroy the capsular HA of the bacteria providing entrance for the phage into the bacterial domain. The entered phage particle still might contain a portion of hyaluronidase that often in case of *Streptococci* gets integrated to the bacterial genome. *Streptococcus pyogenes* genome accommodates genes which produce bacterial hyaluronidase. Most of the strains are known to contain additional phage sequences too in its genome (lysogen) that again encodes for hyaluronidase. Thus, *Streptococcus pyogenes* which has the genes for both bacterial & phage encoded hyluronidase can be referred as the carrier of twins (one of the gene produces bacterial encoded & the other produces phage encoded hyaluronidase)[7,8]. Both the bacterial & the phage encoded hyaluronatelyases act via a β-elimination mechanism on its substrate[9,10]. The bacterial hyaluronidases are exoenzymes which makes use of the N-terminal signal peptide region for transport into the extracellular ambience. In contrast the phage encoded hyaluronidases lack an N-terminal signal peptide sequence and remain in the intracellular ambience but they may get into the extracellular space by residing in the phage particles which are liberated after the lysis of the *Streptococcal* cells[8]. It has been estimated that for a group of 100 *Streptococcus pyogenes* strains only 23 are capable of producing the extracellular hyaluronidase[8]. In *Streptococcus pyogenes* two kinds of bacteriophage hyaluronatelyases have been characterized so far HylP1 & HylP2[9,11]. The major difference between the bacterial & the bacteriophage encoded hyaluronatelyase is the substrate specificity. Contrary, to the bacterial lyases the phage lyases are superlative in substrate specificity i.e. they recognize Hyaluronic acid as their only substrate & do not act upon other structural homologues like Chondroitin-4-Sulphate, Chondroitin-6-Sulphate[9-11]. This reflects on the fact that the phage encoded lyases may be less concerned with the spread of an infection since they don't cleave

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other glycosaminoglycans except HA in turn which shows their dedication in their work of wiping the capsular HA to get access into a bacterial cell. However reports exist revealing their indirect role in an infection (Red rash of Scarlet Fever)[9,12] in which they may allow the inflow of toxins in the host cells. Another distinguishing feature between the bacterial & phage encoded enzyme is the distributive nature of the phage enzyme i.e. they produce longer length oligosaccharide products whereas the bacterial enzymes are highly processive in nature i.e. they produce very small fragments of unsaturated disaccharide products[10,13].

WAY OF PRODUCTION OF HYALURONIDASE

The anabolism of capsular Hyaluronic acid is facilitated by the expression of hasA gene whereas the catabolism of human cell hyaluronic acid is facilitated by the expression of the hylA gene of *Streptococcus pyogenes*. The latter is a dependent variable on function of the serotype & property of the strain. The bacterial hyaluronate lyase is an extracellular enzyme which is exported out from the cell in the fused form having a signal peptide attached to it; cleavage of the signal peptide in the ECM of the bacterial cell produces the active enzyme[7,14]. The proenzyme secreted outside the cell will be influenced by external factors associated with the cell; it may either get transformed to the active enzyme by the cleavage of the signaling peptide or might be activated by external sources like thiol groups of cysteine, cystine, serine or methionine. Antagonistically, it might also get disintegrated or repressed by sources like valine, proline, vitamin B5 (Pantothenic Acid) or the presence of protease[15]. Thus, the proenzymic bacterial hyaluronidase secreted outside the cell might get converted into the active form or might get inhibited / disintegrated depending on the external environment constituents.

HOW DOES HYALURONIDASE WORK?

The exact mechanism by which bacterial hyaluronidase cleaves hyaluronic acid is by a β-Elimination process. Several ways have been proposed and proved in other *Streptococcus* species like *Streptococcus pneumoniae, Streptococcus agalactiae* etc.[13,16,17]. A similar mechanism might be assumed to occur in *Streptococcus pyogenes* hyaluronidase involving proton acceptance and shunting steps. We have modeled the *Streptococcus pyogenes* hyaluronidase and we have found out that *Streptococcus pyogenes* hyaluronidase using homology modeling using *Streptococcus pneumoniae* as the template and have found out that they are 51 % homologous & the residues in the catalytic triad remains conserved, the model has been submitted to Protein Model Data Bank [PMDB(PM0077933)]. Thus, we hypothesize a similar PAD (Proton Acceptance and Donation Mechanism of hyaluronan degradation by hyaluronidase of *Streptococcus pyogenes*. The following steps might happen in the real mechanism:

1) The positively charged residues in the enzyme cleft offer an electrostatic attractive force to the negatively charged substrate HA.
2) The residues in the aromatic patch sense the bond of cleavage in the substrate chain.
3) The hero of the whole picture (Figure 1) (the active site residues enact their role).

3a) the residue Z- interacts with the acidic proton of D- Glucuronic acid.

3b) the residue Y abstracts the acidic proton resulting

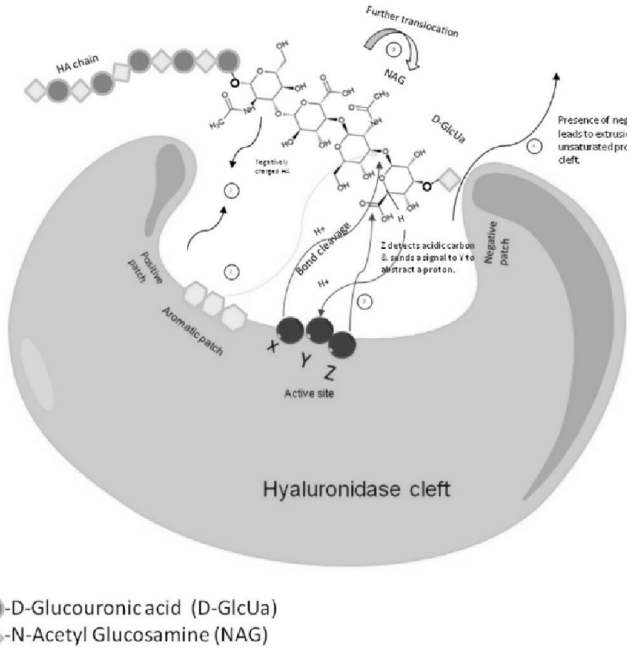

Figure 1 : Hypothetical model for working of Streptococcus pyogenes hyaluronidase

in an unstable intermediate.

3c) the residue X donates a proton to the glycosidic bond oxygen & ultimately the bond linkage is broken.

4) The negative residues towards the other end of the cleft (the one near the products formed) are negative and the product tagged with a net negative charge is extruded from the cleft.

5) The active site residues retrieve their original states via proton exchange with their microenvironment & the substrate marches forward for subsequent rounds of catalysis.

CONCLUSION

Armageddon the battlefield of the good and the evil forces can be correlated to the war between the evil *Streptococcus pyogenes* and the virtuous human cells. Here the sinner (*Streptococcus pyogenes*) can be penalized by whipping it with powerful counter missiles, namely hyaluronidase inhibitors. This ultimately blocks the spread of *Streptococcal* infection. There are many ways to achieve this goal one of this could be designing of inhibitors having stronger anionic nature than D-Glucuronic moiety HA (pK_a>3.2) (Songlin L *et al.*, 2000) such that a competition between the substrate and the inhibitor prevail for the accommodation into the positive patch of the cleft (Figure 2). In this competition if the pK_a of the inhibitor is more, then the winner would be the inhibitor helping the human race to ward off the infectious *Streptococcus pyogenes*. Hyaluronidases are known to be group of neglected enzymes (Kreil G, 1995) in the scientific world, if their potency in causing the *Streptococcus pyogenes* infection is well characterized in future definitely they would turn out to be the hot spots in Designing of drugs towards Streptococcal infections.

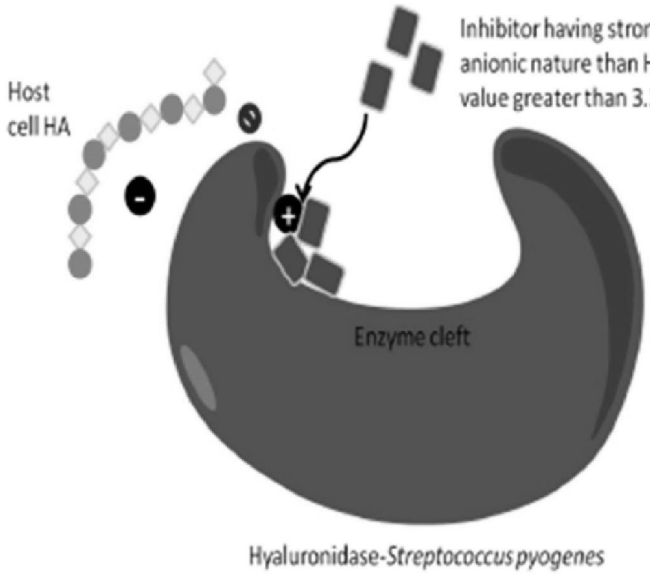

Figure 2 : Hypothetical model on antagonizing the action of Streptococcus pyogenes hyaluronidase

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