Mechanism of hyaluronan binding and degradation: structure of *Streptococcus pneumoniae* hyaluronate lyase in complex with hyaluronic acid disaccharide at 1.7 Å resolution

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Hyaluronic acid (HA) is an important constituent of the extracellular matrix and its degradation has been postulated to contribute to the spread of certain streptococci through tissue [1]. Pneumococci and other streptococci produce hyaluronate lyase, an enzyme that depolymerizes HA; thus, hyaluronate lyase might contribute directly to bacterial invasion [2–5]. Although two different mechanisms for lyase action have been previously proposed, there was no crystallographic evidence to support those mechanisms. Here we report the high-resolution crystal structure of *Streptococcus pneumoniae* hyaluronate lyase (spnHL) in the presence of hyaluronic acid disaccharide, which ultimately provides the first crystallographic evidence for the binding of HA to hyaluronate lyase. This structural complex revealed a key interaction between the spnHL protein and the substrate and supports our previously proposed novel catalytic mechanism for HA degradation based on the native spnHL structure. The information provided by this complex structure will likely be useful in the development of antimicrobial pharmaceutical agents.

The spnHL structure consists of a 361-residue N-terminal domain (α-domain, residues 171–531) and a 347-residue C-terminal domain (β-domain, residues 543–889). The two domains are connected by a short, linker polypeptide of 11 residues (residues 532–541). The N-terminal domain is comprised of 13 α-helices; the helices are arranged into a twisted (α/α) barrel structure as observed in other polysaccharide degrading enzymes such as glucoamylase and alginate lyase. The β-domain consists of 25 β-strands grouped into five antiparallel β-sheets. The detailed description of the native structure has been reported elsewhere [2].

As anticipated, the two disaccharide molecules (HA1 and HA2) are located inside the substrate-binding cleft close to one another but there is no direct interaction between them. Instead, they interact via water-mediated hydrogen bonds. HA1 and HA2 replace four and five water molecules, respectively, known to be present in the native spnHL protein. In the crystal structure, HA1 and HA2 adopt different conformations. In HA2, the sugar ring of NAc2 and UA2 are in the same plane, whereas in HA1 the angle between the planes of sugar rings of NAc1 and UA1 is approximately 45° (Figure 1).

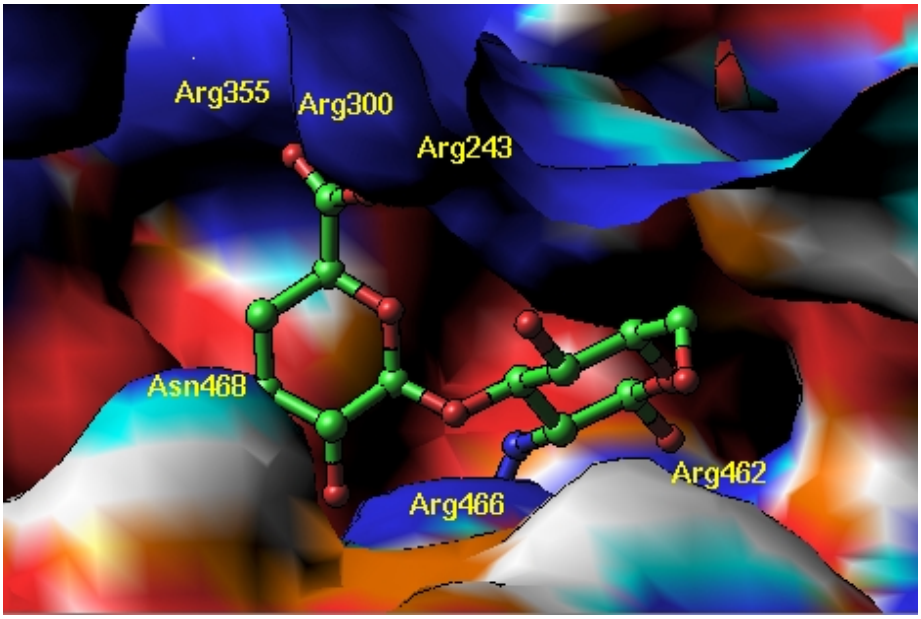

Figure 1: Electrostatic surface potential representation of a part of the carbohydrate binding cleft in the spnHL with bound substrate HA1. The highly positively charged region is represented in blue. Substrate HA1 is shown in ball-and-stick model.

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