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# Presence of Collagen-like Repeats in Bacteriophage–encoded Hyaluronate Lyase

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***Abstract:*** **Gly-X-Y (Gly represents glycine) motif is a collagen-like repeat present at the N-terminal domain of bacteriophage hyaluronate lyase (Phage HL). It addition in providing conformational stability to the protein, it also has non-specific regulatory role in substrate binding as well as in modulation of the enzymatic activity *in vitro*. The presence of Gly-X-Y motif in the prophage genome is a virulence factor of vertebrate origin that arose through lateral gene transfer. On the basis of a number of evidences from our and other groups we hypothesise that the Gly-X-Y repeat might help the Phage HL in spreading infection by adhering at the surface of the human epithelial cell and assist in congregation of phage particles at the site of infection. The Gly-X-Y repeat may also cause polyarthritis by cross-reacting with tissue collagen during the infection of host streptococci. Therefore we propose that group A streptococcus may exploit the Phage HL for the possible pathogenesis by using the cell adherence property of Gly-X-Y collagen repeats. This is a new avenue of research in Phage HLs as the Phage HL has broad substrate degradation propensity.**

***Index Terms:*** **collagen like repeat, enzyme activity, hyaluronic acid, hyaluronate lyase, *Streptococcus***

## I. INTRODUCTION

The Gly-X-Y motif is a collagen-like repeat composed of a glycine residue that recurs in every three amino acid, followed by the positions X and Y, which are often filled by proline residues (Beck and Brodsky, 1998). The glycine and proline residues confer a stable triple helical structure to the mammalian collagen. The collagen-like repeats have been shown to be present in a number of proteins including fibronectin, maltose-binding proteins, as well as in the structural proteins of some bacteriophage and Group A Streptococcus (Smith et al., 1998; Lukomski et al., 2000).

Gly-X-Y motif is also found to be present in the enzyme hyaluronate lyase (HL), a phage tail fibre protein (Figure 1A). The bacteriophage hyaluronate lyases (Phage HLs) are mainly encoded by prophages of *Streptococcus sp.* such as *Streptococcus pyogenes and Streptococcus equi.* Phage HLs are class of enzymes that catalyze the degradation of polymeric hyaluronic acid (HA) into a mixture of smaller unsaturated oligosaccharides, reducing $\Delta HA_{10}$ to $\Delta HA_2$ (HA represents hyaluronic acid) by β-elimination mechanism(Singh et al., 2014a). The enzyme has been reported to have substrate specificity for HA, though it has limited ability to degrade chondroitin 6-sulfate as well as dermatan sulphate (Singh et al., 2014a).

The HL genes found in the bacteriophage genome shows a high degree of similarity with each other. However, the major difference seems to be the deletion or addition of a 102-bp fragment consisting of a region encoding a collagen-like motif, Gly-X-$Y_{10}$ motif (10 repeats of Gly-X-Y) repeating units localized at the N-terminal region (Hynes and Ferretti, 1989). Therefore, significant differences exist in the amino acid residues present at the N-terminal domain, mainly because of the presence of Gly-X-Y motif and protein sequences, which are substantiated by displaying 80% similarity among the amino acid residues present at the C-terminus. Based on the presence or absence of collagen-like domain, Phage HLs can be classified as either the HylP-type that contains a collagen-like repeat sequence or the HylP2-type that is devoid of the above-mentioned region. Phage HLs, HylP and SEQ2045, are grouped under HylP-type; HylP1, HylP2, and HylP3 are grouped under HylP2-type (Hynes et al., 1995; Baker et al., 2002; Smith et al., 2005; Lindsay et al., 2009).

## II. ROLE OF GLY-X-Y MOTIF IN SUBSTRATE REGULATION AND STABILITY

The presence of collagen motif in bacteria and viruses underlines the importance of collagen as a structural motif in nature. Several studies have suggested that the Gly-X-Y motif mediates protein trimerization or elongation in bacteria or

bacteriophage HLs (Charalambous et al., 1988; Caldentey et al., 2000; Xu et al., 2002; Sylvestre et al., 2003).

The Gly-X-Y repeat domains in certain proteins, including host defense proteins, make them adaptable to a range of protein assemblies (Ramshaw et al., 1998). It was speculated that amino acid residues within these repeats have the potential expected. Conversely, Gly-X-Y motif was observed to interact with the calcium ions, as well as showing non-specific interaction with polymeric HA suggesting a non-specific regulatory role of Gly-X-Y motif in HA binding as well as modulating the enzymatic activity of Phage HLs *in vitro* (Singh et al., 2014a; Singh et al., 2014b).

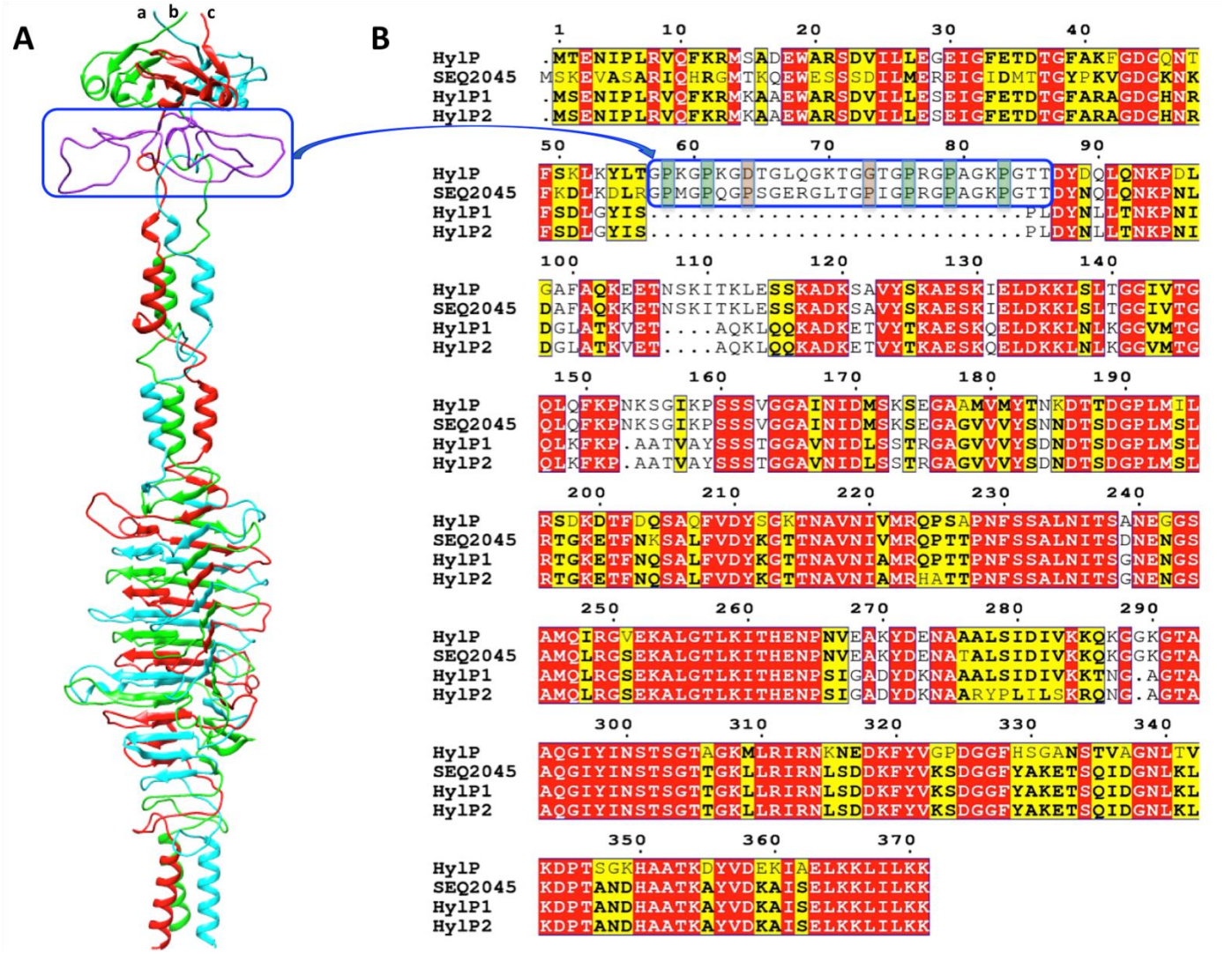


**Figure 1: Gly-X-Y motif of Phage HLs.** (A) Modelled three-dimensional figure of HylP Phage HL generated by the Swiss model based on the template of HylP1 (PDB ID: 2C3F). The generated trimeric assembly was formed by PISA and viewed through Chimera. (B) The multiple sequence alignment of Phage HLs. Blue box represents the Gly-X-Y motif. Green and brown shaded box represents the conserved and non-conserved proline respectively. Similar residues are shown in yellow boxes; the red boxes represent identical amino acid residues while the amino acid residues with different properties have no boxes.

to undergo posttranslational hydroxylation and may be involved in the stabilization of trimeric structure (Stern and Stern, 1992). The structural study on Phage HLs was reported only for the HylP2-type and no structural information was available on HylP-type protein containing Gly-X-Y collagen motif at the N-terminal domain (Smith et al., 2005; Mishra et al., 2009). According to the crystal structure of HylP2-type HLs, the catalytic domain of the enzyme is significantly far away (~100 Å) from its N-terminal region, so the role of Gly-X-Y collagen motif on the functional activity of the HylP-type HL was least

In case of Phage HLs, Gly-X-Y motif codes of a minimum size are needed for the formation of stable collagen triple helix. However, Gly-X-Y motif only provides the conformational stability to phage HLs which may be due to the presence of proline at the conserved or non-conserved position in HylP-type phage HL (Figure 1B) and have no role in trimerization as this region is found missing from the HylP2 domain (Mishra et al., 2006; Singh et al., 2014a; Singh et al., 2014b).Additionally, these collagen repeats also provide elongated structure to Phage HL (Shukla et al., 2015). Therefore,

the series of 10 repeats of Gly-X-Y motif significantly influenced the overall functional activity of the HylP-type of Phage HL. The result steal importance of being new role of Gly-X-Y collagen repeat in calcium binding and modulating the enzymatic activity of Phage HL, asearlier report suggest that calcium ions influence the activity of HLs by activating the hyaluronic acid (HA) conformation for proper binding to the active site cleft of bacterial HLs (Akhtar et al., 2006). Also, Gly-X-Y motif present adjacent to the N-terminal globular α/β domain of SEQ2045and HylP, might provide the structural constraint needed for the substrate selection, anchoring, targeting, etc. Therefore, future elucidation of the structure of HylP-type phage HLs represents an interesting scientific venture.

## III. ROLE OF GLY-X-Y MOTIF IN PATHOGENESIS: A NEW AVENUE FOR RESEARCH

The role of bacterial HLs in human pathogenesis is known since long (Cole et al., 2011) . An earlier study suggested the role of Phage HLs to be confined in the breaking of the host HA capsule for lysogenization (Hynes and Walton, 2000; Baker et al., 2002). Our recent study on Phage HLs suggested that the *Streptococci* might use Phage HLs in conferring virulence and disease pathogenesis, which indicates that the role of Phage HLs is not just limited to bacterial lysogenization but also acts as a potential virulence factor during pathogenesis (Halperin et al., 1987; Broudy et al., 2001, 2002; Lindsay et al., 2009; Singh et al., 2014a). The presence of Gly-X-Y motif in the prophage genome is an example of putative lateral gene transfer (Stern & Stern, 1992). This is considered to be a virulence factor of vertebrate origin that might facilitate adhesion of the phage HL enzyme to the collagenous tissues of the host.

Plethora of reports suggested that collagen binds with the cell adhesion receptors that participated in attachment to the host human cells (Wayner & Carter, 1987; Ramachandran, 1988; Hasty et al., 1992; Ruoslahti, 1996; Napper et al., 2006). Most importantly Scl protein that contains a large region composed of collagen repeats participated in Group A Streptococcus (GAS) attachment to human epithelial cells, which subsequently aided an increase in the bacterial molecules that contributed towards the pathogenesis process (Hasty et al., 1992). Taking this into account, there is a high probability that Gly-X-Y repeats might facilitate the adherence of Phage HLs to the surface of human epithelial cell upon bacterial lysis or induction (Broudy et al., 2001), and thereby congregate the phage HLs at large number at the site of infection and help in spreading of the infection by GAS. In fact, HylP2-type Phage HL might infect with different mode (possibly by binding trough their C-terminal domain) to various cell surface receptors of host or forming the functional fibril during phage infection (Mishra & Bhakuni, 2009; Shukla et al., 2015).

There also has been considerable speculation that GAS-induced autoantibodies plays a significant role in many other human diseases including rheumatoid arthritis and systemic lupus erythematosus, which is mainly due to the pathology of collagen within the diseased sites and the presence of anticollagen antibodies in patient sera (Gioud et al., 1982; Gibofsky et al., 1998). Moreover, the autoimmune diseases can be induced in case of experimental animals after exposure to collagen (Cremer et al., 1998). The discovery of a widely distributed extracellular GAS protein and presence of collagen repeats in Phage HLs with structural features similar to that of human collagen, might add another dimension to the autoimmunity considerations and induction of antibodies against collagen (Halperin et al., 1987). Therefore, a possible role of collagen-like repeat (found in the Bacteriophage HLs) in causing human diseases could be the induction of antibodies, which might cross-react with the tissue collagen and result in polyarthritis that is often associated with rheumatic fever. Also, the bacterial HL might utilize the Phage HL collagen repeats for molecular mimicry of the host proteins, which is a common strategy adopted by bacterial pathogens to interfere with and exploit host processes (Albert & Inman, 1999).

Understanding the structural parameters and functionalities of HylP-type Phage HL shall further enhance our understanding about this useful polysachharide degrading enzyme containing collagen repeats, both in terms of catalysis and its role in assisting Phage HL for possible human pathogenesis.

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