Inhibition of Mammalian Glycoprotein YKL-40: Identification of Physiological Ligands

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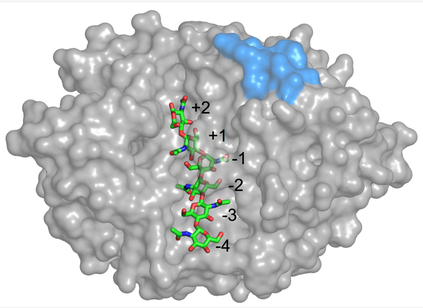
Abstract

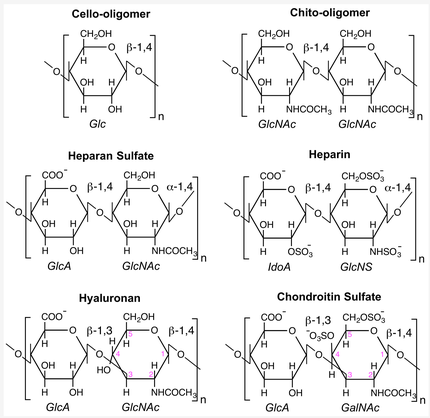
YKL-40 is a non-catalytic mammalian glycoprotein and known biomarker associated with progression, severity, and prognosis of chronic inflammatory diseases and a multitude of cancers. Despite this well-documented association, conclusive identification of the lectin's physiological ligand, and accordingly biological function, has proven experimentally difficult. Chitin has been identified as preferred ligand by binding affinity studies; however, the natural presence of chitin in the human body has not yet been documented. Here, we consider polysaccharides including glucose, hyaluronan, heparan sulfate, heparin, and chondroitin sulfate, as well as collagen-like peptides as potential physiological ligands for YKL-40. Molecular dynamics (MD) simulations resolve the molecular-level recognition mechanisms, as several of these potential ligands appear to bind YKL-40 analogous to chitin. Further, we calculate the ligand binding free energy of the hypothesized ligands with YKL-40 to address the thermodynamic preference relative to chitin. Our results suggest that chitin and hyaluronan preferentially bind to YKL-40 over collagen, and hyaluronan is likely the preferred physiological ligand. Collagen binds in two locations at the surface of YKL-40 and may be related to its role in fibrillar formation. Finally, heparin non-specifically binds at the surface of YKL-40 as predicted from structural studies. Overall, YKL-40 likely binds to many natural ligands in vivo, but its concurrence with physical maladies may be related to the associated increase in hyaluronan. As a potential therapeutic target, these fundamental insights enable the rational design of YKL-40 antagonists to inhibit this action.

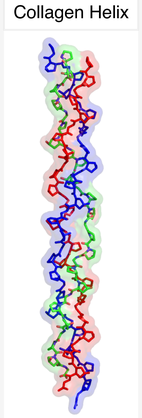
YKL-40 – Chitin-binding glycoprotein



- Mammalian YKL-40 is classified as a glycoside hydrolase family 18 chitinase (PDB ID: 1HJW) [1]
- The primary carbohydrate binding region has nine binding subsites within a cleft lined with aromatic residues for stacking interaction-mediated binding.
- A putative heparin binding site located within a surface loop has also been suggested (blue).

- Our objective is to understand the biological relevance of YKL-40 in humans by investigating its interactions with physiological carbohydrates and proteins. From our study, we obtain molecular-level insights that aid in designing robust solutions for diseases associated with YKL-40.



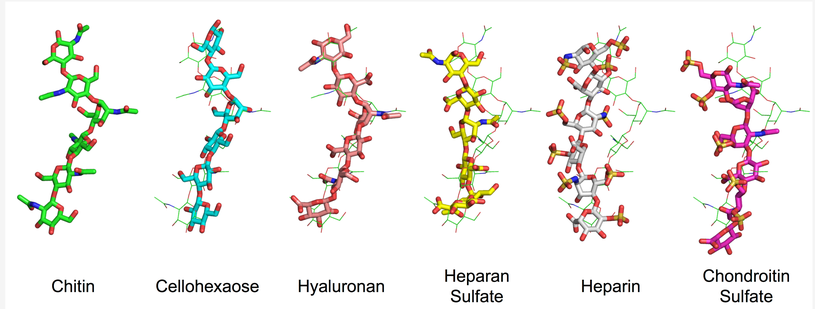


Tools & Methods

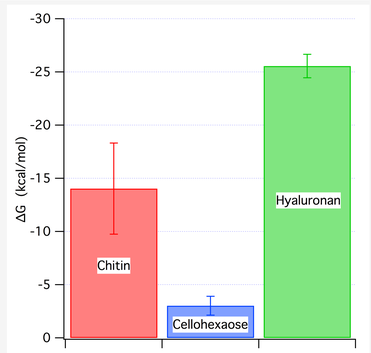
- PatchDock molecular docking
- Molecular dynamics simulations using CHARMM and NAMD
- Free Energy Perturbation with replica exchange molecular dynamics (FEP/λ-REMD)[3]
- Umbrella sampling potential of mean force calculations with native contacts.

Protein-polysaccharide Binding in YKL-40

- All six oligomers were docked in the binding site and simulated for 250 ns.
- Of the six considered oligomers, only chitin, cellohexaose and hyaluronan remained bound in the pocket. Heparan sulfate, heparin and chondroitin sulfate were ejected into the the bulk.

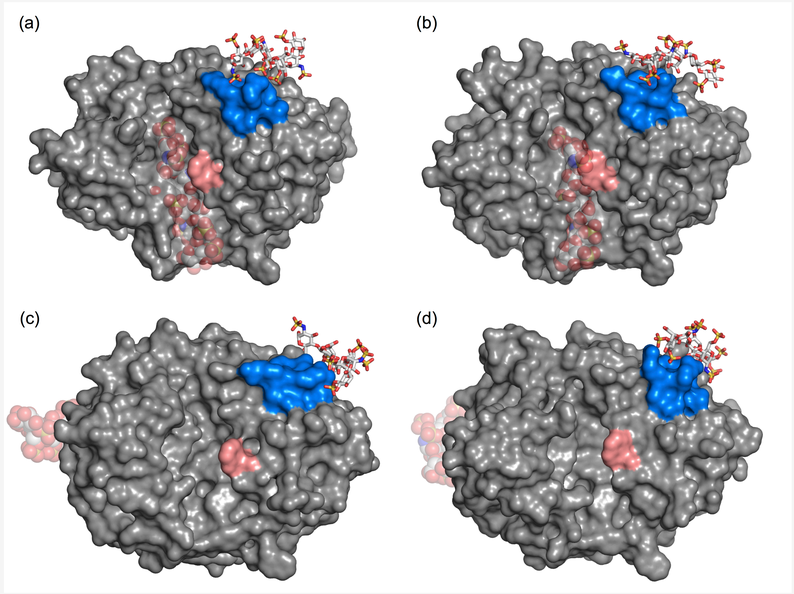


- Absolute ligand binding free energies of YKL-40 for hexamers of chitin, glucose and hyaluronan were calculated using FEP/λ-REMD.
- Chitin binds to YKL-40 with similar affinity as to family 18 chitinases.
- Hyaluronan binds more similar to chitin, and with rather high affinity, exhibiting its potential characteristics in inhibition of YKL-40.

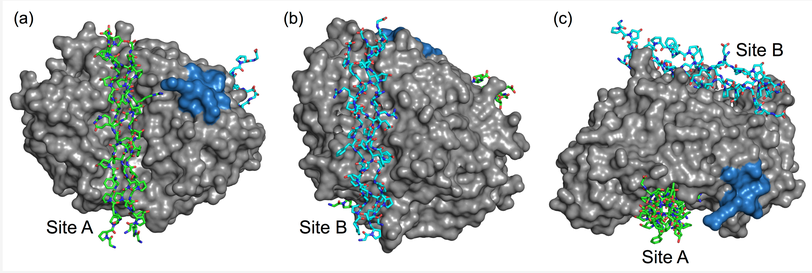


Putative Heparin Binding Site

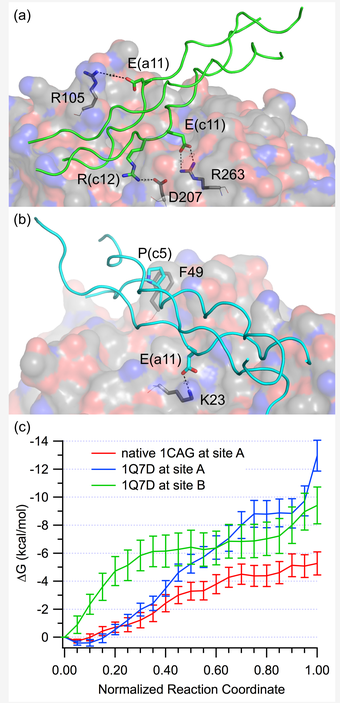
- After being forced out of primary carbohydrate binding site, heparin finds the putative heparin binding site on the surface of YKL-40 (a domain of **GRRDKQH** with high basic amino acid density). When initially positioned in bulk, heparin again migrates to this putative heparin binding site.



Collagen Binding in YKL-40



- YKL-40 specifically binds to three types of collagen fibers and has effects on the fibril formation process,[2] however binding sites and mechanisms are still unknown.
- We suggest there are **two** potential binding sites for collagen-like peptides on YKL-40, according to criteria of molecular surface shape complementarity.
- Four collagen model peptides were studied in complex with YKL-40 at both the binding sites.
- Collagen-like peptide model with the integrin binding domain GFOGER (1Q7D) stably binds with both sites. Both sites have almost the same affinity for collagen model 1Q7D.
- Additional salt-bridges formed in 1Q7D (not formed in native 1CAG) account for higher free energy of binding.



Conclusions

- MD simulations and free energy calculations overwhelmingly suggest polysaccharide ligands, in particular chitin and hyaluronan, are preferential physiological ligands of YKL-40.
- Non-specific interaction of heparin with the putative heparin-binding site, as suggested by previous studies, was confirmed by MD simulation.
- Detection of potential binding sites for collagen and comparison of dynamics and affinities over different collagen peptide models provided insights to functions of YKL-40 in collagen fibril formation.
- Increased serum levels of both hyaluronan and YKL-40 are associated with physical maladies and the affinity of YKL-40 for multiple ligands, but hyaluronan in particular, suggests their coexistence as physiologically relevant phenomenon.

References and Acknowledgements

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