

Isothermal Titration Calorimetric and Computational Studies on the Binding of Chitooligosaccharides to Pumpkin (*Cucurbita maxima*) Phloem Exudate Lectin

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Abstract:

The interaction of chitooligosaccharides [(GlcNAc)_{2–6}] with pumpkin phloem exudate lectin (PPL) was investigated by isothermal titration calorimetry and computational methods. The dimeric PPL binds to (GlcNAc)_{3–5} with binding constants of $1.26\text{--}1.53 \times 10^5 \text{ M}^{-1}$ at 25 °C, whereas chitobiose exhibits approximately 66-fold lower affinity. Interestingly, chitohexaose shows nearly 40-fold higher affinity than chitopentaose with a binding constant of $6.16 \times 10^6 \text{ M}^{-1}$. The binding stoichiometry decreases with an increase in the oligosaccharide size from 2.26 for chitobiose to 1.08 for chitohexaose. The binding reaction was essentially enthalpy driven with negative entropic contribution, suggesting that hydrogen bonds and van der Waals' interactions are the main factors that stabilize PPL–saccharide association. The three-dimensional structure of PPL was predicted by homology modeling, and binding of chitooligosaccharides was investigated by molecular docking and molecular dynamics simulations, which showed that the protein binding pocket can accommodate up to three GlcNAc residues, whereas additional residues in chitotetraose and chitopentaose did not exhibit any interactions with the binding pocket. Docking studies with chitohexaose indicated that the two triose units of the molecule could interact with different protein binding sites, suggesting formation of higher order complexes by the higher oligomers of GlcNAc by their simultaneous interaction with two protein molecules.