Characterisation of the Structural Requirements for the Immunomodulatory Surfactant Protein D (SP-D) to Bind to Maltose Based Ligands

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Introduction: Surfactant protein D (SP-D) is an important part of the airway innate immune system, responsible for clearance of invading pathogens. As a calcium dependent collagenous lectin, SP-D belongs to the collectin family and uses its carbohydrate recognition domain to interact with ligands on microbes such as bacteria and viruses. An understanding of how the lectin domain of human SP-D interacts with selected saccharides, with different glycosidic bond linkages and configurations (1->3, 1->4 and 1->6) and in addition saccharide lengths, would enhance the understanding of the structural requirements required for binding of SP-D to pathogens, which potentially could be used for therapeutic purposes. Materials and methods: A half-maximal inhibitory concentration (IC50) assay with a recombinant fragment of human SP-D O(rfhSP-D) binding to mannan was employed. The concentration at which 50% of inhibition of the rfhSP-D binding occurred was determined using the saccharides maltose, manNAc, β-isomaltotriose (1->6), β-maltotriose (1->4), β-maltoheptaose (1->4), β-cellotriose (1->4), β-laminaritriose (1->3) and β-galactose. Non-linear regression was used to calculate IC50 values (mM) from sigmoidal curves and results reported as mean±standard deviation (SD). Results:The relative IC50 values displayed a trend of β-isomaltotriose (1.32+/-0.06) > β-maltotriose (1.51+/-0.13) > β-maltoheptaose (1.97+/-0.11) > manNAc (3.60+/-0.12) > maltose (4.42+/-0.30) > β-cellotriose (5.02+/-0.42) > β-laminaritriose (8.61+/-0.20) >> galactose (52.50+/-8.42). Discussion: The general trend of the highest affinity binding of β-isomaltotriose, β-maltotriose and β-maltoheptaose were in accordance with values from the literature, as well as the preference of rfhSP-D to manNAc over maltose and a high IC50 value for galactose. Data from crystallographic and computational studies showed that β-isomaltotriose and β-maltotriose bind through an internal glycosyl residue resulting in a high binding affinity for rfhSP-D. Factors such as saccharide length and the configuration of the glycosidic bond linkage, making sugars such as β-cellotriose and β-laminaritriose more linear showed higher IC50-values. Conclusion: The results from the study highlights that the binding of a ligand is not only determined by the presence of hydroxyl groups but other structural factors such as the length and configuration also have an influence on the binding. Using the knowledge gathered about the ligand specificity of the rfhSP-D CRD as a basis for future studies of more complex sugar-containing ligands, it may be possible at one point to predict whether or not SP-D treatment would be beneficial based on the structure of the targeted ligand/pathogen.

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