Immobilized Glycosidases: Interesting Tools For The Elucidation Of Carbohydrate-Protein Interaction In Biological Processes.

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Introduction

Glycoconjugates are involved in biological recognition processes and changes in glycan profiles correlated with several pathologies. They also takes part in host-pathogens interaction. Enzymatic tools based on the use of endo- and exo-glycosidases constitute a promising strategy to identify the nature and function of the carbohydrates of glycoproteins and glycolipids present in clinical samples and pathogen organisms. This will contribute to the study of diseases associated to biological process mediated by glycans. The use of immobilized glycosidases enables their reuse and ease their separation from the reaction medium.

Objectives

Study of the performance of immobilized *A. oryzae* β-galactosidase and *C.ensiformis* α-mannosidase in the deglycosilation of glycoproteins and *Fasciola hepatica* lysate.

Materials and Methods

glycosidases were immobilized onto agarose and nanoparticles activated with cyanate ester groups. Deglycosilation was performed at room temperature for 24 hours. After removal of the glycosidase, the deglycosilated protein and the released monosaccharides were separated by size exclusion chromatography. Changes in affinity towards specific lectins of the deglycosilated glycoproteins were evaluated and released monosaccharaides identified.

Results and discussion

Degalactosilation of asialfetuin and demannosidation of lactoferrin using immobilized glycosidases was achieved and the feasibility of their reuse proved. Release of galactose and mannose in the degalactosilation and demannosidation process respectively was confirmed by TLC and HPLC. A loss of more than 60% in the affinity for ConA was observed for the demannosidated lactoferrin. Degalactosilation of *Fasciola hepatica* lysate showed a loss in the affinity for PNA of 50% while affinity for ConA remained unchanged. Conversely demannosidation of the lysate arose a loss of affinity for ConA of 50% without changes in affinity for PNA, confirming the selectivity of the glycosidases.

Conclusion

Immobilized β-galactosidase and α-mannosidase proved to be useful tools in the selective deglycosilation of glycoconjugates, encouraging the immobilization of other glycosidases of frequent use in glycobiology.

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Key words

Enzymes, Glycosidases, Immobilization, Deglycosilation.