XVIII Congreso Nacional de Biotecnología y Bioingeniería
León, Guanajuato 23 al 28 de junio 2019
Resumen de Trabajos Libres

INTERACTION PROPERTIES BETWEEN PEGYLATED PROTEINS AND A MODIFIED RESIN BY ISOTHERMAL TITRATION CALORIMETRY (ITC) AND FTIR

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Keywords: PEGylation, HIC, Isothermal titration calorimetry.

Introduction. PEGylated proteins are an increasing important class of therapeutic drugs due to their improved pharmacokinetic characteristics and solubility over their corresponding native forms (1). PEGylation is the covalent attachment of one or more polyethylene glycol (PEG) molecules to a protein. Despite the many advantages of PEGylated drugs, one of the major challenges is the purification step after the chemical reaction (2). The main purpose of this project was to determine the nature of chemical interactions between a modified resin with PEG 5000 g/mol and PEGylated proteins and evaluate the thermodynamic parameters involved in the adsorption and resolution of PEGylated Sepharose-6B for the purification of proteins by HIC.

Methods. Sepharose 6B (SEP6B) PEGylation reaction was done according to Hernández-Martínez (3) employing methoxy-PEG of 5000 g/mol. Quantification of the attached PEG was performed with an indirect measure of remaining PEG on reaction solution. Iodine/barium chloride method was used, as reported by Gong *et al* (4). RNase A and Lysozyme were used as model proteins for PEGylation reactions, conducted according to Cisneros-Ruiz M (5). An ATR-FTIR spectrophotometer was used to confirm PEGylation of SEP6B using a Spectrum One ATR-FTIR (Perkin Elmer, Massachusetts, USA). The purification process was carried out according to Hernández-Martínez (3) employing 1 mL Tricorn columns on an Äkta Pure FPLC (GE Healthcare, Uppsala, Sweden). Microcalorimetric measurements were performed on a MicroCal VP-ITC (Malvern, Worcestershire, UK). All experiments were performed at 25ºC. Results were analyzed with Origin™ program (Malvern, Worcestershire, UK).

Results. Modification of SEP6B was performed by covalent attachment of PEG molecules via nucleophilic addition of methoxy-PEG to the reactive oxyrane group of activated resin. Quantification of attached PEG was determined indirectly, by measuring residual in the reaction solution, resulting in 0.0805 ± 0.0055 mmol PEG/g SEP6B. FTIR analysis was performed to demonstrate chemical modification of SEP6B (**Fig. 1**).

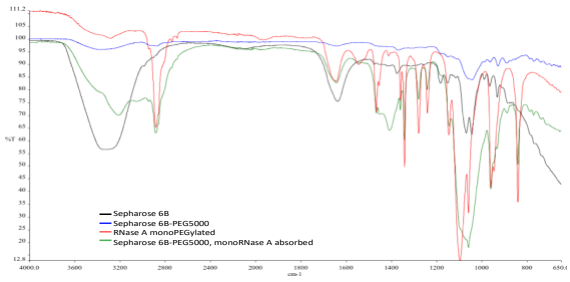

Fig. 1. FTIR spectra of SEP6B (black), SEP6B-PEG5000 (blue), monoPEGylated RNase A (red) and SEP6B-PEG5000 with absorbed monoPEGylated RNase A (green).

A thermodynamic analysis was performed in order to comprehend the interaction mechanism involved in the efficiency of the modified SEP6B. Isothermal titration calorimetry (ITC) enabled the determination of the affinity, the binding enthalpy and the stoichiometry in a single titration experiment at constant temperature. It monitors the produced (exothermic) or absorbed (endothermic) heat during binding (46) (**Fig. 2**).

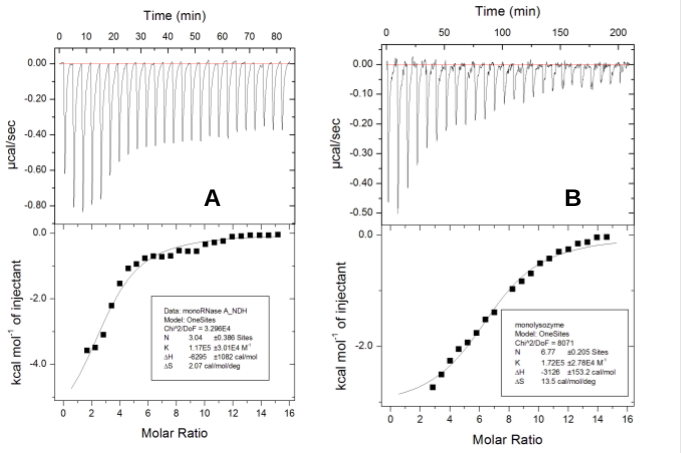

Fig. 2. Isothermal Titration of monoPEGylated model proteins with PEG5000. A) RNase A; B) lysozyme.

Conclusions. The presence of PEG molecules attached to hydroxy groups on the resin was demonstrated by a significant decrease in the intensity of -OH signals and its characteristic absorption bands in IR spectra. FTIR analysis demonstrated the chemical modification of the support and lead to a broad approach of binding between protein and resins. Lysozyme demonstrated favorable binding affinity with PEG5000, correlated with its retention behavior on the PEGylated chromatographic support. Isotherms of PEGylated proteins show a reduction of enthalpy forces (ΔH) compensated with an arrangement in the entropy (ΔS) by a decreased mobility restriction. Binding constant of monoPEGylated proteins were higher than native proteins, showing a stronger interaction with PEG5000 molecules of modified resin. FTIR and microcalorimetric analyses contribute meaningful information in processes designed for a commercial application and its future impact in the pharmaceutical area.

Acknowledgement. To CONACyT Ciencia Básica 182537

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