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Boric acid gel enrichment of glycosylated proteins in human wound fluids☆

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ABSTRACT

The enrichment of glycosylated proteins by glycocapturing materials plays a pivotal role for the investigation of polysaccharide containing proteins in disease pathogenesis. Hence, we investigated a boric acid gel as a binding material for glycoprotein enrichment. The bovine proteins alpha-1-acid-glycoprotein (A1AG) and alpha-2-HS-glycoprotein (fetuin A) were spiked in human chronic wound fluids and were subsequently enriched by a boric acid gel affinity chromatography (BAGAC). The enrichment efficiency was evaluated by western blot analysis and mass spectrometry. Additionally, glycoproteins of human wound fluids from diabetes mellitus patients with chronic foot ulcers were analyzed after BAGAC enrichments. In total 104 glycoproteins were identified, with reported glycosylation sites. 60 proteins were detected in at least 2 out of 3 biological replicates and were used for quantitative analysis between the bound and unbound fractions. Almost 80% of these glycoproteins were more prominent in the bound fraction. Only 2 glycoproteins revealed higher spectral counts in the flow through fraction compared to the bound fraction. These findings demonstrate the capability of the BAGAC material to enrich glycosylated proteins from complex human wound fluids.

1. Introduction

The human wound fluid (WF) is one of the most complex human body fluids containing secreted proteins. These fluids have highly time dependent protein compositions for different steps in wound healing. This dynamic variation reveals information about the wound status.

Stagnation of chronic wounds in different phases of the wound healing process induces the interest in protein biomarker discovery in wound fluids. Known from glycoproteomic studies of diseases, such as cancer or other genetic diseases, glycosylated proteins are in several cases involved in pathogenesis, because of non- or mis-glycosylated proteins or through over- or underexpression of glycoproteins [1–4]. Analysis of human foot ulcers from *diabetes mellitus* patients already showed that some post translational glycosylated matrix metalloproteinases (MMP) are overexpressed in wound fluids [3].

Glycosylation of proteins is one of the most common post translational modifications (PTM) in mammalians. More than

Abbreviation: BAGAC, Boronic Acid Gel Affinity Chromatography; SCX, Strong Cation Exchange; MudPIT, Multi-dimensional Protein Identification Technology.

☆ The ethics committee of the Ruhr-University Bochum, Germany, approved the study protocol.

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50% of all proteins are supposed to be glycosylated [5]. The investigation of post translational glycosylated proteins is of high interest for biomarker discovery. The main approach to investigate glycoproteins is the use of carbohydrate capturing materials for affinity enrichment. Lectins are widely used to enrich glycosylated proteins. These highly specific carbohydrate binding proteins were found not only in plants, but also in animals and bacteria and can be used to bind specific glycoforms [6–8], such as N-glycosylated or O-glycosylated proteins, or to bind different glycoforms in a multi lectin affinity chromatography (MLAC) approach [9]. Highly specific glycoprotein enrichment might be advantageous. However, for investigation of the whole glycoproteome, less specific strategies might be useful.

Boronic acid binds carbohydrates with 1,3- or 1,4-dioles. Immobilized on agarose it can be used to enrich glycosylated proteins [10]. This material was also combined with lectins to improve the binding capacity in a boronic acid lectin affinity chromatography (BLAC) [11]. Xu et al. functionalized gold coated silica wafer with boronic acid for an improved glycoprotein enrichment [12].

Numerous different enrichment methods for glycoproteins indicate that such techniques are still in need of improvement. Therefore, we investigated a cross polymerized boronic acid material for its useful application of glycoprotein capture in human wound fluids.

2. Methods and materials

2.1. *Sample collection*

3 human wound fluid samples were collected from diabetes mellitus patients showing chronic foot ulcer from the University Hospital Bergmannsheil (Bochum, Germany).

For sample collection PVA sponges were applied to the wound bed to withdraw the exudates. Soaked sponges were moistened with 2 ml of a Roche Complete protease inhibitor cocktail solution, inhibiting serine, cysteine and metalloproteinases (Penzberg, Germany). To collect the wound fluid, sponges were carefully centrifuged at 125 ×*g*. This step was repeated twice. Purified solutions were frozen with liquid N_2 and stored at −80 °C until further analysis. Protein concentrations were determined with a BCA protein assay (BCA Protein Assay Kit, Pierce Chemicals, Rockford, IL).

The study protocol was approved by the ethics committee of the Ruhr-University Bochum, Germany.

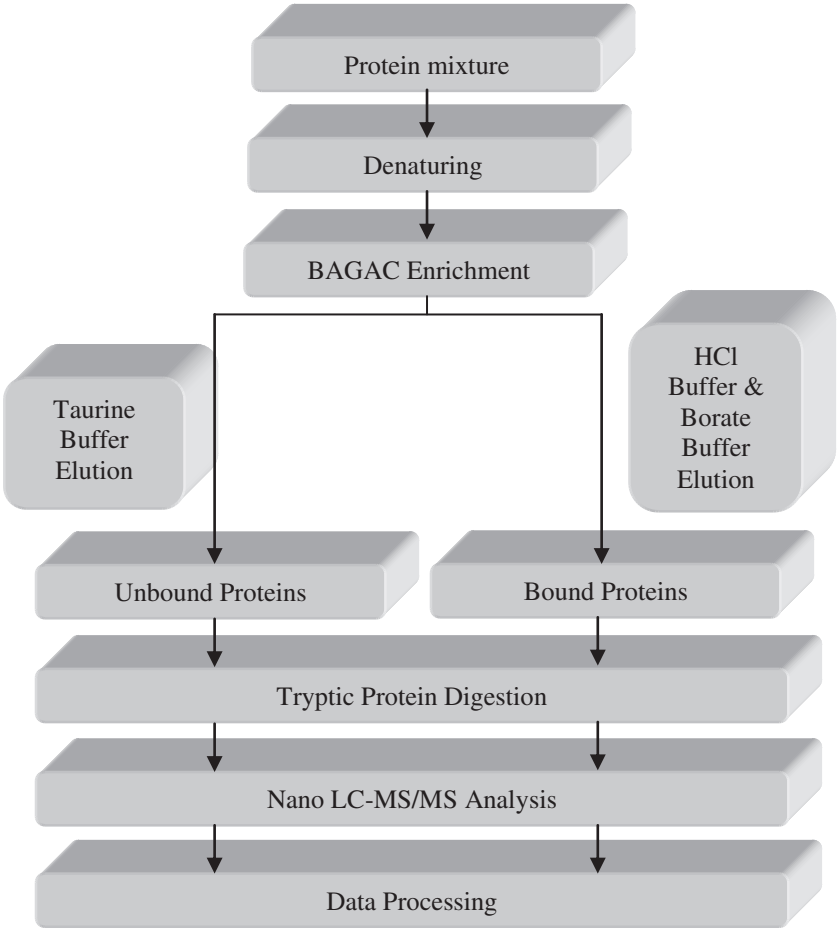

Fig. 1 – BAGAC glycoprotein enrichment workflow.