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Synthesis of adsorbents with dendronic structures for protein hydrophobic interaction chromatography

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ABSTRACT

Here, we introduced a new technology based on the incorporation of dendrons—branched chemical structures—onto supports for synthesis of HIC adsorbents. In doing so we studied the synthesis and performance of these novel HIC dendron-based adsorbents. The adsorbents were synthesized in a facile two-step reaction. First, Sepharose 4FF (R) was chemically modified with polyester dendrons of different branching degrees *i.e.* third (G3) or fifth (G5) generations. Then, butyl-end valeric acid ligands were coupled to dendrons *via* ester bond formation. UV–vis spectrophotometry and FTIR analyses of the modified resins confirmed the presence of the dendrons and their ligands on them. Inclusion of dendrons allowed the increment of ligand density, 82.5 ± 11 and 175.6 ± 5.7 μmol ligand/mL resin for RG3 and RG5, respectively. Static adsorption capacity of modified resins was found to be ~60 mg BSA/mL resin. Interestingly, dynamic binding capacity was higher at high flow rates, 62.5 ± 0.8 and 58.0 ± 0.5 mg/mL for RG3 and RG5, respectively. RG3 was able to separate lipase, β-lactoglobulin and α-chymotrypsin selectively as well as fractionating of a whole proteome from yeast. This innovative technology will improve the existing HIC resin synthesis methods. It will also allow the reduction of the amount of adsorbent used in a chromatographic procedure and thus permit the use of smaller columns resulting in faster processes. Furthermore, this method could potentially be considered as a green technology since both, dendrons and ligands, are formed by ester bonds that are more biodegradable allowing the disposal of used resin waste in a more ecofriendly manner when compared to other exiting resins.

1. Introduction

A major need in the biotech industry is the isolation and purification of proteins from complex mixtures where protein downstream processing may account for up to an 80% of the final product total cost. Hence, the development of novel isolation and purification methodologies or the optimization of the current standardized protocols to achieve the highest purities and yields, raises mayor attention in the design of bioprocesses. In this context, chromatographic methods are the most used techniques for purification of proteins due to the high recovery and purities achieved. Among these, size exclusion chromatography (SEC), hydrophobic interaction chromatography (HIC), ion-exchange chromatography (IEX), reverse phase chromatography (RPC) and affinity chromatography (AC) are the most common used protein separation chromatographic techniques [1,2].

HIC is a powerful and widely used technique for purification of proteins [3,4]. It is a key methodology when purification of monoclonal antibodies [5–7] is required. It is often used in the final polishing step of downstream processes as it can easily remove high-molecular weight aggregates [8]. HIC relies on the interaction between the hydrophobic regions on the surface of the biomolecules and the ligands on the support under high concentration of salts *i.e.* ammonium sulfate or sodium chloride [3]. There are numerous commercially available adsorbents with different types of hydrophobic ligands *i.e.* butyl, octyl and phenyl groups. However, one of the major drawbacks in HIC is the low ligand density that impacts directly on the adsorption capacity of resins. Furthermore, the methods used to activate the support materials might be

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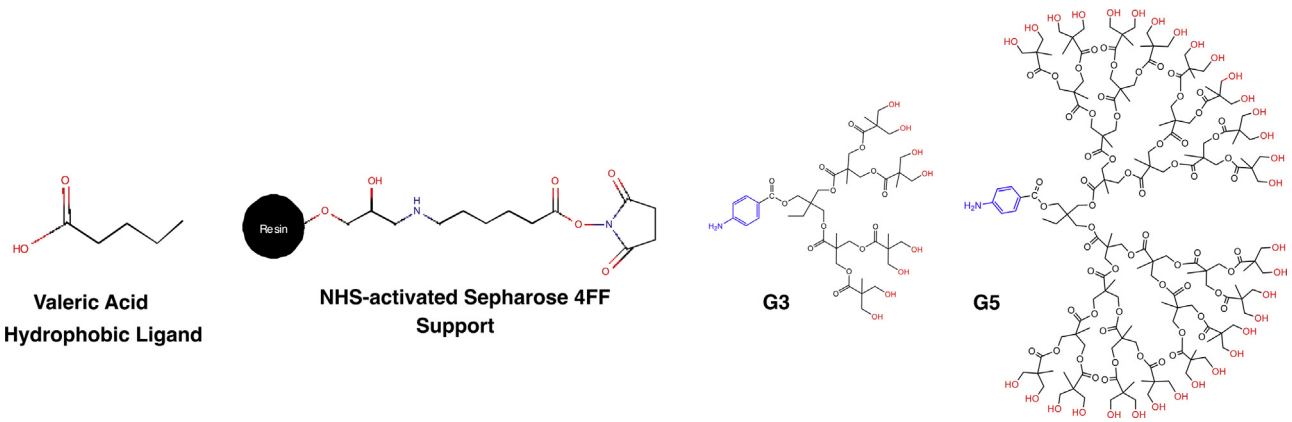

Fig. 1. Chemical compounds used for the synthesis of the hydrophobic adsorbents. Chemical structures from left to right are valeric acid, resin, and dendrons of third (G3) and fifth (G5) generations. Marvin was used for drawing, displaying and characterizing chemical structures, substructures and reactions, Marvin 15.8.3.0, 2015, ChemAxon (http://www.chemaxon.com).

tedious or are sometimes based on bromide-derived compounds, which are toxic to environment. For this reasons, new HIC matrix functionalization methodologies and options are needed which may also provide further advantages to this commonly used unit operation.

Synthesis of chromatographic media is a challenging task. It requires a precise selection of the chemistry to be used and the reproducibility of the obtained ligand density between batches may become a drawback in this process. Commercially, the most used method for activation of solid supports is achieved through the incorporation of epoxy groups onto the matrix that are subsequently reacted with the desired ligand [9]. This strategy provides a general method to attach ligands for their use in a great variety of separation techniques such as IEX, HIC, RPC or AC and it governs the market. Another method, but not preferable to epoxy activation, is the cyanogen bromide activation method [10]. However, it has major disadvantages such as reagent toxicity, the addition of undesired ion exchange interactions, and the instability of the *N*-substituted isourea linkage that causes improper attachment of the ligands. In contrast, here for the first time, we developed a method for immobilization of ligands based on esterification reactions that yielded stable bonds as resins were reused many times as it will be described later. However, further studies may involve immobilization of ligands based on amide bonds that are even more stable than ester bonds. Nonetheless, the innovation presented here could be considered as a green technology as it is friendly to the environment in terms of waste disposal as ester bonds are more biodegradable than traditional resins based on epoxy reactions.

In this instance, dendrons are a class of synthetic macromolecules that might be tailored for a specific application due to their well-defined structure and flexibility [11]. These polymers possess a branched structure anchored to a focal point of any chemistry, which emanates radially toward the molecular periphery ending in any desired chemical moiety that, in turn, can be functionalized for a specific application [11]. So far, dendritic structures have been widely exploited as nano-carrier systems for drug delivery [12] and in the design of DNA microarrays [13]. There are as well some reports regarding modification of stationary phases with dendrimers [14,15], but not for protein purification.

In this work, we have extended the application of dendritic chemical structures to the synthesis of novel chromatographic adsorbents in an innovative way through their incorporation onto chromatographic matrixes and the subsequent covalent attachment of ligands by formation of ester bonds onto the dendron molecules. The branched architecture of the dendritic molecules allows the attachment of a higher amount of ligands onto the resin and the generation of hydrophobic clusters throughout it. This results in multiple interaction sites with stronger hydrophobic interactions between the proteins and the adsorbent. This work deals with the synthesis of dendronized adsorbents for protein HIC and their performance in the separation of model proteins and a whole proteome.

2. Experimental

2.1. Chemicals

Third generation (G3, Cat. No. 767263) and fifth generation (G5, Cat. No. 767239) bis-MPA (2,2-Bis(hydroxyl-methyl) propionic acid) polyester dendrons manufactured by Polymer Factory Sweden AB (Stockholm, Sweden) used for the synthesis of the adsorbents were bought from Sigma-Aldrich (St. Louis, MO, USA). NHS-activated Sepharose 4FF (Cat. No. 17-0906-01) was acquired from GE Healthcare (Uppsala, Sweden). Baker's yeast (*Saccharomyces cerevisiae*) was acquired locally in Monterrey, México. Model proteins including bovine serum albumin (BSA, Cat. No. A4503), lipase (LP, L3126) from porcine pancreas, β-lactoglobulin (β-Lac, Cat. No. L3908) from bovine milk, and bovine pancreatic α-chymotrypsin (α-Quym, Cat. No.C4129) were bought from Sigma-Aldrich. *N*,*N*-dimethylformamide (DMF, Cat. No. 227056), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, Cat. No. E7750), toluene (Cat. No. 244511), ethanol (EtOH, Cat. No. 02860), *N*-hydroxysuccinimide (NHS, Cat. No. 130672), dimethylaminopyridine (DMAP, Cat. No. 107700), valeric acid (VA, Cat. No. 240370), ethyl acetate anhydrous (Cat. No. 270989) and 2-propanol(iso-propOH, Cat. No. 650447) of HPLC grade were also purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetone (Cat. No. AH010) and methanol (MeOH, Cat. No. AH230) of HPLC grade were purchased from Honeywell Burdick and Jackson (Morris Plains, NJ, USA). *p*-Toluensulfonic acid (PTSA, Cat. No. 89762) was bought from Fluka Analytical (St. Louis, MO, USA). All other chemicals used were all at least of analytical grade.

2.2. Catalyst synthesis for ester reactions

4-(dimethylamino)pyridinium 4-toluenesulfonate (DPTS) was prepared by reacting equivalent amounts of anhydrous PTSA and DMAP. The latter was dissolved in 40 mL of anhydrous toluene and added to PTSA. The suspension was filtrated, washed with toluene and dehydrated in vacuum. The resulting powder was used in the ester synthesis.