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Short communication

Synthesis and characterization of biospecific adsorbents containing glucose, usable to retain concanavalin A

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Abstract

The synthesis and characterization of poly(butadiene-hydroxyethylmethacrylate–epichlorhydrin–D-glucose) (PB HEMA ECH-D-Glu) and poly(butadiene–hydroxyethylmethacrylate–epichlorhydrin-L-glucose) (PB–HEMA–ECH-L-Glu) hydrogels are presented. The applicability of these products was studied in order to establish their stereospecific capacity in the retention of concanavalin A.

1. Introduction

The synthesis of biospecific adsorbents used in affinity chromatography to purify biological macromolecules such as proteins, antibodies and enzymes is an important area of research [1–3]. Lectins, proteins capable of specific interaction with carbohydrate-containing substances, possess the ability to agglutinate erythrocytes of specific blood groups and different types of cells [4,5]. Concanavalin A (Con A), a lectin isolated from jack bean (*Canavalia ensiformis*), has become the most widely investigated lectin because it exhibits a series of remarkable properties. It has been used as haemaglutinin [6,7], for studies of cell surface and cell division, to examine the nature of the carbohydrate residues responsible for blood-group specificity and to distinguish between malignant or abnormal and normal cells. This glucose-binding lectin has been isolated and purified to homogeneity by affinity chromatography on biospecific adsorbents: cross-linked dextran gel (Sephadex) [8–12], Sepharose as adsorbent followed by elution with methyl-α-D-gluco(or manno)pyranoside [5] or using D-glucosamine immobilized on diol-bonded silica [13].

In this paper, we describe the synthesis and characterization of affinity materials and report on their utility in the biospecific retention of Con A. Epichlorhydrin was used for the introduction of reactive oxirane groups into a poly(butadiene–hydroxyethylmethacrylate) (PB–HEMA) synthetic matrix, followed by coupling of glucose (D- or L-).

2. Experimental

2.1. Materials and analysis

Epichlorhydrin was obtained from Riedel-de

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Haën, D-glucose from Anedra and L-glucose and concanavalin A from Sigma.

Determinations of hydroxyl and oxirane equivalents were carried out using the acetyl and pyridinium chloride methods, respectively [14].

Microdetermination of glucose (D- or L-) was carried out by spectrophotometry using the phenol–sulfuric acid method [15].

IR spectra were recorded on a Nicolet 5-SXC spectrometer. Scanning electron microscopy (SEM) was performed on a Phillips SEM 501 B instrument at the laboratories in the Centro de Investigaciones de Materiales y Metrología (CIMM).

Swelling indices (Sw) were determined in water, after 48 h, as the ratio of the swollen (V_s) and dried (V_d) volumes of samples, respectively ($Sw = V_s/V_d$).

Glass transition temperatures (T_g) were determined on a Mettler TA 3000 DSC system at the laboratories in the Instituto de Desarrollo Tecnológico para la Industria Química (INTEC).

Lectins were measured using a modified version of the Lowry method [16] and UV–Vis spectra were measured with a Shimadzu UV 260 recording spectrophotometer.

2.2. Preparation of biospecific affinity adsorbents

Activation of PB-HEMA matrix

The synthesis of the PB–HEMA matrix was carried out by graft copolymerization, using polybutadiene resin (PB), hydroxyethylmethacrylate (HEMA) and benzoyl peroxide. The activation of the synthesized matrix was performed using epichlorhydrin under alkaline conditions [17]. The matrix (PB–HEMA) and the corresponding epoxy-activated product poly(butadiene–hydroxyethylmethacrylate–epichlorhydrin) (PB–HEMA–ECH) have been purified and characterized previously [17].

Coupling of glucose (D- or L-) to the activated matrix

The biospecific adsorbents for concanavalin A lectin were prepared as follows: the epoxy-activated PB–HEMA–ECH matrix (1 g) was swollen in dimethylformamide (DMF) (10% w/v) for 17 h. This wet gel was mixed with a solution of D-glucose (or L-glucose) (2 g) in 0.1 *M* NaOH (48.84 ml) and $NaBH_4$ ($4 \cdot 10^{-3}$ g) was added. The mixture was incubated at 45°C for 8.5 h on a shaker in a water-bath.

The PB–HEMA–ECH–D-Glu and PB–HEMA–ECH–L-Glu hydrogels were washed extensively with water, 0.2 *M* sodium acetate buffer (0.5 *M* NaCl) at pH 4.0 and 0.2 *M* borate buffer (0.5 *M* NaCl) at pH 8.0. They were then studied by IR spectrometry, SEM and differential scanning calorimetry (DSC).

A portion of each product (0.1 g) was hydrolysed with 2 *M* HCl in dioxane–water (8:2) at 100°C for 23 h and the amount of carbohydrate covalently bound to the activated matrix was subsequently determined.

2.3. Biospecific affinity chromatography

A portion of each biospecific adsorbent obtained (0.1 g) was incubated with solutions of Con A (10 ml) at different concentrations (0.2, 0.4 and 0.8 mg/ml) in 1 *M* NaCl, $1 \cdot 10^{-3}$ *M* $CaCl_2$ and $1 \cdot 10^{-3}$ *M* $MnSO_4$ for 16 h at room temperature with stirring. All determinations were carried out in triplicate.

The amount of remaining and bound Con A per gram or millilitre of hydrogel was determined. The possible retention of Con A by epoxy-activated PB–HEMA–ECH matrix was also measured.

3. Results and discussion

3.1. Preparation of biospecific affinity adsorbents

Activation of PB–HEMA matrix

The PB–HEMA matrix, a yellowish powder, able to swell in water, contained 3.93 mequiv. of hydroxyl groups per gram of dry gel, which confers a hydrophilic character on the matrix and

provides non-ionic reactive functional groups capable of undergoing chemical modifications.

The reaction carried out between epichlorhydrin and the matrix involved its activation and attainment of a product with a high content of terminal oxirane functional groups, 0.74 mequiv. per gram of dry gel.

Coupling of glucose (D- or L-) to the activated matrix

The synthesis of PB–HEMA–ECH–D-Glu and PB–HEMA–ECH–L-Glu hydrogels involved the coupling of glucose (D- or L-) to the activated matrix under alkaline conditions, for which sodium borohydride was added in catalytic amounts to minimize side-reactions (decoloration, oxidative attack and decomposition) of the carbohydrates. The reactions were carried out using DMF as solvent in which the epoxidized matrix was highly swellable.

Both biospecific adsorbents, PB–HEMA–ECH–D-Glu and PB–HEMA–ECH–L-Glu, presented the same swelling index in water, $Sw = 4.0$. Samples were hydrolysed and the sugar contents were determined. The PB–HEMA–ECH-D-Glu gel was found to contain 40.08 μmol of D-glucose per gram of dry gel or 10.02 μmol of D-glucose per ml of swollen gel and therefore it was established that 65.11 μmol of L-glucose had been attached per gram of dry gel or 16.25 μmol of L-glucose had been attached per ml of swollen gel. It is known [18] that in the structure of the hydrogels, the carbohydrate binds preferentially by the primary hydroxyl of C-6, the most reactive hydroxyl group.

Bands at 3040, 1150–1160, 1050–1150 and 1030 cm^{-1} were observed by IR spectrometry in the spectra, attributable to the stretching vibrations of primary and secondary hydroxyl groups, secondary hydroxyl groups of the glucose, ether and primary alcohol groups, respectively.

The microphotographs obtained from products by SEM showed a porous surface needed for the inclusion of biological macromolecules. This can be seen in Fig. 1.

The T_g of the products PB–HEMA, PB–HEMA–ECH and PB–HEMA–ECH–D-Glu, obtained from DSC curves, with values of 49.96, 41.10 and 35.20, respectively, suggested that, at room temperature, the products are rigid and hard. The fact that the T_g values do not rise with chemical modification by incorporation of epichlorhydrin and the ligand on the cross-linked PB–HEMA matrix could indicate that they occur on the surfaces in absence of cross-linkages. In general, the incorporation of aliphatic side-chains decreases the T_g value [19]. This is in accordance with the results obtained, leading to less rigid structures.

3.2. Biospecific affinity chromatography

In the binding to glucosidic residues, Con A requires Mn^{2+} and Ca^{2+} ions to form a quaternary structure and create the binding points to the carbohydrates. A transition metal such as Mn^{2+} is needed to bind Ca^{2+} and both are necessary for glucose or mannose binding [20].

Tables 1 and 2 summarize the results for the retention of Con A using the PB–HEMA–ECH–D-Glu and the PB–HEMA–ECH–L-Glu adsorbents, respectively, which differ in the relative capacity to retain the lectin. The absence of non-specific interactions (hydrophobics) was corroborated by using the epoxidized matrix, whose capacity to retain Con A was zero.

As can be observed in Table 1, the PB–HEMA–ECH–D-Glu adsorbent retained 49.0 ± 0.4 mg of Con A lectin per gram of dry gel or 12.3 mg of Con A lectin per ml of swollen gel. The retention of Con A using PB–HEMA–ECH–L-Glu indicated a saturation of 4.1 mg of Con A lectin per ml of swollen gel (see Table 2).

In general, in affinity chromatography, the level of saturation of a ligand bound to the matrix is hyperbolically related to the concentration of proteins or biological macromolecules [21]. Consequently, to reach a high degree of gel saturation, the solution that contains the protein must be as concentrated as possible. This hypothesis was corroborated from the results for the retention of Con A lectin using the PB–HEMA–ECH–D-Glu hydrogel obtained.

Fig. 2 shows the structures of D-glucose and L-glucose. D-Glucose is a monosaccharide that contains binding points with Con A lectin

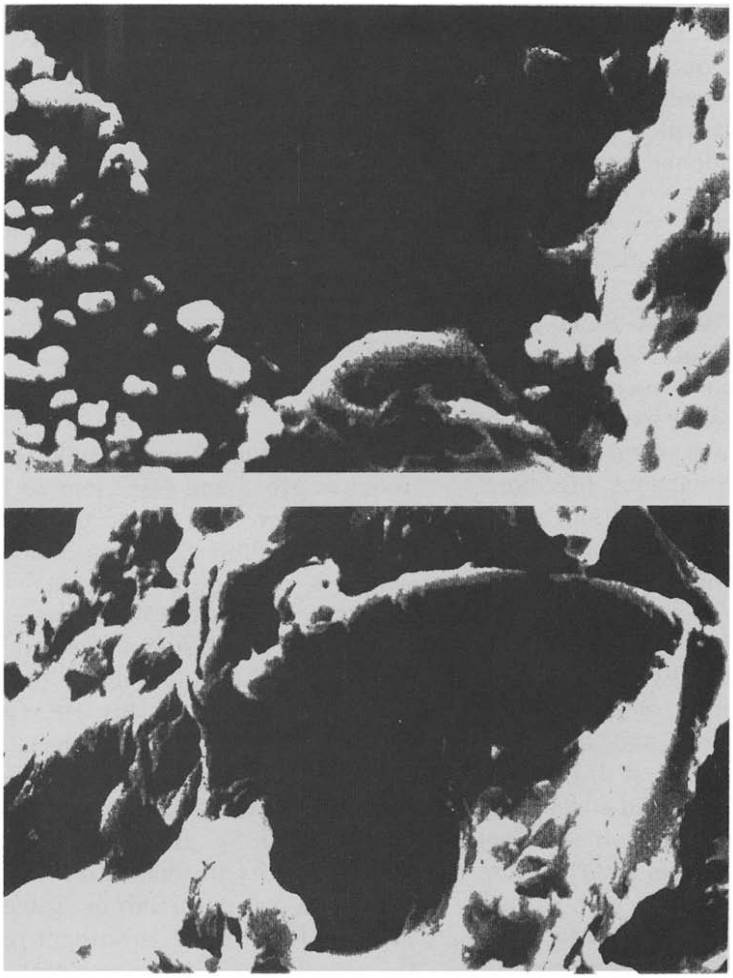

Fig. 1. Microphotographs of the hydrogels obtained by SEM.

through the hydroxyl groups of C-3, C-4 and C-6 of the pyranosic ring. The specificity of the interaction indicates the preference for the "α" anomer of C-1 and the equatorial form for the hydroxyl groups of C-3 and C-4. Any modification of these hydroxyl groups, such as a change in configuration, drastically decreases or abolishes the interaction with the protein [22].

Table 1
Retention of Con A using PB–HEMA–ECH–D-Glu adsorbent

Concentration of Con A (mg/ml)	Con A per gram of dry gel (mg)	Con A per ml of swollen gel (mg)
0.2	16.7 ± 0.8	4.2
0.4	25.6 ± 0.9	6.4
0.8	49.0 ± 0.4	12.3

Table 2
Retention of Con A using PB–HEMA–ECH–L-Glu adsorbent

Concentration of Con A (mg/ml)	Con A per gram dry gel (mg)	Con A per ml of swollen gel (mg)
0.2	13.3 ± 0.8	3.4
0.4	13.6 ± 0.8	3.4
0.8	16.5 ± 1.4	4.1

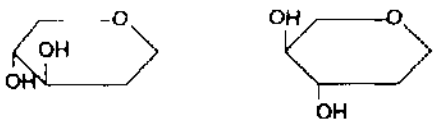

Fig. 2. Structures of D-glucose and L-glucose based on configuration of hydroxyl groups at C-3 and C-4 [23].

As seen in Fig. 2, the hydroxyl groups of C-3 and C-4 of L-glucose show changes in configuration with respect to D-glucose [23].

The binding points with the Con A lectin in D-glucose could be displayed in a specific binding form, as indicated by the results obtained using PB–HEMA–ECH–D-Glu adsorbent, with high retention of lectin. A change in the configuration for L-glucose clearly decreases the retention of lectin using PB–HEMA–ECH–L-Glu adsorbent, corroborating the importance of the arrangement of the hydroxyl groups of C-3 and C-4 in an equatorial form in PB–HEMA–ECH–D-Glu hydrogel.

4. Conclusions

The synthesis of PB–HEMA–ECH–D-Glu and PB–HEMA–ECH–L-Glu hydrogels has been achieved. The activation of the PB–HEMA matrix yielded an epoxidized product with a high content of terminal oxirane functional groups (0.74 mequiv. per gram of dry gel). This value is comparable to those for activated matrices obtained by other workers [24] from natural polysaccharides and epichlorhydrin.

On the other hand, the amount of carbohydrates bound to the activated matrix (40.08 μmol of D-glucose per gram of PB–HEMA–ECH–D-Glu dry gel and 65.11 μmol of L-glucose per gram of PB–HEMA–ECH–L-Glu dry gel) may be assumed to represent typical incorporation values for monosaccharides under the coupling reaction described, as 3.3–66 μmol of ligand per gram of dry gel or 1–20 μmol of ligand per ml of rehydrated gel is suitable for most adsorbents [25].

The retention of Con A using PB–HEMA–ECH–D-Glu (49.0 ± 0.4 mg of Con A lectin per gram of dry gel) was comparable to those attained using commercial gels such as agarose-β-D-glucose obtained from macromolecules from natural sources, that retained 10–20 mg of Con A lectin per ml of rehydrated gel [26].

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