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Surface energy components of a dye-ligand immobilized pHEMA membranes: Effects of their molecular attracting forces for non-covalent interactions with IgG and HSA in aqueous media

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Abstract

In the present paper, we report the study of the adsorption behaviour of human immunoglobulin G (IgG), human serum albumin (HSA) and polyethylenimine (PEI) onto surfaces of Procion Green HE-4BD (PG) immobilized poly(hydroxyethylmethacrylate) (pHEMA) membranes. The adsorption behaviour of the IgG and HSA onto surfaces of the PG–PEI complexed membrane was also studied. Surface wettability and hydrophilicity of all the membranes were investigated by static contact angle measurements. The measurements of the contact angle to various test liquids, i.e., water, glycerol, formamide, diiodomethane (DIM) and ethylene glycol on the investigated membranes were made by sessile drop method. In accordance to the Young equation, the smaller the surface tension of the test liquid, the smaller becomes the contact angles measured on all the investigated membranes surfaces. The highest contact angles were obtained with water, whereas ethylene glycol gave the lowest contact angles for all the tested membranes. Component and parameters of the surface free energy of all the investigated membranes were calculated from measured contact angle values using two methods (the geometric mean by Fowkes and acid–base by van Oss). HSA adsorption was enhanced after complexation of PEI with the immobilized dye-ligand. The adsorption of proteins and PEI significantly changed both the contact angles and component of surface free energies of the investigated membranes.

Keywords: Dye-ligands; Affinity membranes; HSA; IgG; PEI; Adsorption; Contact angle; Surface energy

1. Introduction

Protein adsorption plays a major role in a variety of important technological and biological processes. An ideal affinity membrane for chromatographic application should adsorb specifically a target protein from biological fluids. The performance of an affinity membrane depends on its surface properties. The surface properties of an affinity membrane may affect the adsorption capacity and its behaviour by controlling adsorption of proteins present in the fluids [1–5]. In particular, protein adsorption depends on the surface composition and morphology of the affinity membrane [6–11]. There are reports that the amount of adsorbed proteins on a chromatographic matrix surface is quantitatively changed, depending on the type of ligand molecules used. Selective adsorption of a protein on a chromatographic material is a very complex process, which can be determined by several factors. Among these, the hydrophobic and polar character, the chemical structure of immobilized ligand and the electrostatic interactions of the protein molecules with matrix surface and with each other are the most important [3,12,13]. Therefore, the surface properties of an affinity matrix should be characterized to explain its interactions with proteins. The hydrophobic and polar character can be determined by contact angles of test liquids on these membranes. There are many methods to measure contact angles. The sessile drop technique is the most common due to its simplicity and it has been widely employed for polymer surface energy determination [14,15].

For laboratory- and large-scale separation purposes, textile dyes are used as pseudo-specific affinity ligands after immobilization on polymeric supports for affinity separation of bio-molecules. Proteins, peptides, nucleic acids and even small molecules (e.g., bilirubin) have been separated using dye-ligands from biological fluids [16]. Each dye-ligand molecule

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has different functional groups such as $–SO_3H$, $–NH_2$, =NH, –COOH, –OH and several aromatic rings for ionic, polar and hydrophobic interaction with various bio-molecules [17,18]. The initial protein adsorption on a matrix should be governed by self-possessions of the intermolecular interactions between the surface amino acid side chains of protein and the adsorbing surface as a function of separation distance (i.e., residue–surface interactions) and inter-alia interactions in the protein tertiary structure (residue–residue interactions). These microscopic-scale interactions can be subdivided into Lifshitz–van der Waals (LW) and Lewis acid–base (AB) contributions and used to predict the free energy of binding and structural organization of the system [19–21].

In the present study, Procion Green HE-4BD as a dye-ligand was immobilized on the poly(hydroxyethylmethacrylate) (pHEMA) membrane via nucleophilic substitution reaction under alkaline conditions. In order to test the affinity of dye-ligand with biological macromolecules, the adsorptions of IgG and HSA onto these surfaces have been studied because the nature of binding mechanisms of the reactive dyes varies significantly from protein to protein and from dye to dye. Polyethylenimine (PEI) has been chosen as a model polycationic polymer because it strongly interacts with negatively charged dye-ligand and will interact more strongly with the negatively charged proteins. After complexation of immobilized dye-ligand with PEI, the interactions of dye-ligand–PEI complex with IgG and HSA were studied under identical experimental conditions. The surface properties of all the investigated membranes were determined by contact angles measurements. The contact angles data were analyzed using two representative methods. The surface energy parameters of all the investigated membranes were determined from those methods.

2. Experimental

2.1. Materials

Immunoglobulin G (IgG) and human serum albumin (HSA) were supplied from Sigma Chemical Co. (St. Louis, MO, USA) and used as received. 2-Hydroxyethylmethacrylate (HEMA) was obtained from Fluka AG (Switzerland), distilled under reduced pressure in the presence of hydroquinone and stored at 4 °C until use. α-α′-Azoisobutyronitrile (AIBN), Procion Green HE-4BD (PG; Reactive Green 19), PEI (MW 25,000), glycerol, ethylene glycol (EG), formamide and diiodomethane (DIM) were obtained from Sigma Chem. Co. All other chemicals were of analytical grade and were purchased from Merck AG (Darmstadt, Germany).

2.2. Synthesis of pHEMA membrane

The membrane synthesis was achieved by mixing a phosphate buffer solution (0.1 M, pH 7.0, 3.0 ml) with 2-HEMA monomer (2.0 ml) containing 20 mg AIBN. Following the nitrogen flash, the solution was poured into a round glass mould (diameter: 9.0 cm), sealed and exposed to UV for 1.0 h at ambient temperature. The nitrogen atmosphere was maintained during the UV irradiation. After polymerization period, the resultant product was washed with distilled water. The pHEMA membrane was cut into circular pieces (diameter: 0.75 cm) with a perforator and left in the wet state at 4 °C.

2.3. Dye-ligand immobilization onto pHEMA membrane

Procion Green HE-4BD was covalently immobilized onto pHEMA membrane via the nucleophilic substitution reaction between the chloride of its triazine ring and hydroxyl groups of the pHEMA under alkaline conditions. Procion Green HE-4BD (300 mg) was dissolved in distilled water (10 ml), and transferred to NaCl solution (1.0 M, 60 ml) in which pHEMA membrane disks (6 g) were equilibrated for 1 h. After this period, sodium carbonate solution (3.2 M, 30 ml) was added to the medium (about pH 10) and heated at 80 °C for 4 h in a sealed reactor. After the reaction period, the solution cooled down to the room temperature and pHEMA membrane disks were washed several times with distilled water, 2.0 M NaCl and 10% methanol. The PG immobilized pHEMA membranes were stored at 4 °C until use.

2.4. Complexation of PEI with immobilized dye-ligand

The effect of pH on the complexation of PEI with the immobilized dye-ligand was investigated in a batch system at 25 °C. In total 50 ml of PEI solution (4.0 mg ml^{-1}) with different pH values (in the pH range 3.0–8.0, adjusted with HCl and NaOH) were incubated with pHEMA or pHEMA–PG membranes in the flasks agitated magnetically at 200 rpm for 1 h. The PG and PEI complexation experiments were carried out at pH 6.0, as described above for obtaining the effects of PEI initial concentration by changing in the range 1.0–8.0 mg ml^{-1}. The concentration of the PEI in the resulting solution was determined at 233 nm using a spectrophotometer.

2.5. Proteins adsorption experiments

Adsorption of IgG and HSA from solutions on the membranes was studied at various pHs's, in either acetate (7.5 ml, 50 mM, pH 4.0–5.5), in phosphate (7.5 ml, 50 mM, pH 6.0–8.0) or carbonate buffer (7.5 ml, 50 mM, pH 8.5–9.0). Adsorption experiments were conducted in stirrer cells at 25 °C, for 2 h while continuous stirring. The initial concentration of IgG and HSA was 2.0 mg ml^{-1} for each set experiment. In order to determine the adsorption capacities of membranes, the concentration of IgG and HSA in the medium were varied between 0.5 and 2.0 mg ml^{-1}. The adsorption experiments were carried out for IgG at pH 6.0 and for HSA at pH 5.0. The amounts of adsorbed IgG and HSA on the tested membranes were determined by measuring the initial and final concentrations of solutes within the adsorption medium. Calibration curves were prepared using IgG and HSA as standard (0.1–2.0 mg ml^{-1}). The concentration of proteins was measured at 280 nm by using a double beam UV–vis spectrophotometer (Shimadzu, Tokyo, Japan, Model 1601).