Mutual Citation

Cibacron blue F3GA-attached 2μm non-porous monodisperse silicas for affinity chromatography

Shen Li, Xiong Bohui, Cong Runzi and Wang Junde

Dalian Institute of Chemical Physics, The Chinese Academy of Science, Dalian 116012,China

Abstract

Non-porous monodisperse silica (NPS), 2μm in diameter, was modified with 3-aminopropyltriethoxysilane for immobilized of Cibacron Blue F3GA, a packing of NPS-ACB for high performance affinity chromatography was obtained. Up to 2 mg of Cibacron Blue F3GA could be attached to 1 ml of NPS beads. There was no obvious leakage of dye from NPS-ACB. The adsorption of lysozyme varied with pH value and ionic strengths. Furthermore, the column could be used for separation and preparation of lysozyme from hen egg white. In addition, rapid separation of α-, β-,γ-globulin is also described.

Key words : high performance affinity chromatography, non-porous monodisperse silica, Cibacron Blue F3GA, lysozyme, α-globulin, β-globulin, γ-globulin

Introduction:

Affinity chromatography is a well known technique for the purification of biopolymers. Conventional soft gel affinity media, owing to its unstable mechanical character, can not be used for rapid separation, which causes low mass and bioactivity recovery in separation process. With the development of high performance affinity chromatography (HPAC), silica[1], metal-oxides[2] and polymers[3,4] have been widely used as affinity media. Since the successful application of non-porous monodisperse silica in affinity chromatography in 1988[5], the use in reversed-phase chromatography[6], ion-exchange chromatography[7] and hydrophobic chromatography[8] has been described.

In this study we used 2μm non-porous monodisperse silica (NPS) as matrix, Cibacron Blue F3GA as ligand, to produce an affinity packing. Experiments have been carried out to determine the interaction between Cibacron Blue F3GA and several proteins and enzymes.

Experimental

Apparatus and Chemicals

Shimadzu LC-10AD liquid chromatograph with SPD-10A UV-VIS Detector (Japan), 7125 Injector (Rheodyne), Chromatographic work station (Dalian Institute of Chemical Physics), 721 UV-Vis Spectrophotometer

Cibacron Blue F3GA (Sigma), 3-aminopropyltriethoxysilane (APS, Fluka), Tris (Fluka), lysozyme (Shanghai Institute of Biochemistry α, β-, γ- (-globulin (Serva).

Preparation of NPS-ACB

NPS was activated by acid treatment and dehydrated under a reduced pressure of 2mm Hg and 453K for 6h.The reaction procedure was carried out after cooling the silica to room temperature. In case of derivatization of the silica in toluene, the solvent was dried with sodium wire, and freshly distilled, and then added to the silica under vacuum, in order to allow the solvent to wet completely the surface of non-porous monodisperse particles. After that some 3-

aminopropyltriethoxysilane was added. The reaction mixture was heated under reflux for 24h. The modified silica was extracted in toluene and trichloromethane for 2 days in order to remove the reaction components. The modified silica was stored under anhydrous conditions until use.

The modified silica was suspended in 0.1 mol/L sodium phosphate, at pH 8.0, containing 0.5 mol/L KCl and Cibacron Blue F3GA. The reaction was carried out at 60˚C for 3h. The immobilized dye matrix was washed in turn with 0.1 mol/L sodium phosphate, methyl alcohol-water (1:1), methyl alcohol, and water until the washing supernatants were colorless.

The Amount of Immobilized Dye

16~60 mg NPS-ACB was solubilized in 5ml NaOH (1mol/L). The amount of dye bonded was determined by measuring the change in absorption at 620nm.

pH Stability Test

NPS-ACB was suspended in 50mmol/L Tris-HCl solutions containing 0.5 mol/L KCl at different pH values and shaken for 24h.The solution was centrifuged and the supernatant was examined for ligand leakage spectrophotometrically at 280nm.

Chromatographic Procedures

NPS-ACB was placed into a stainless-steel column(4.6×50mm I.D.) using the downward slurry technique in water. Mobile phase was 50mmol/L Tris-HCl. Eluent was 50mmol/L Tris-HCl containing 1mol/L KCl and 20% glycol (v/v).

Results and Discussion

The Character of NPS-ACB

NPS with 2μm particle size (R.S.D.<5%) was a gift of University of Petroleum, China. The surface area was $4m^2/g$ [8] as shown in Fig. 1.

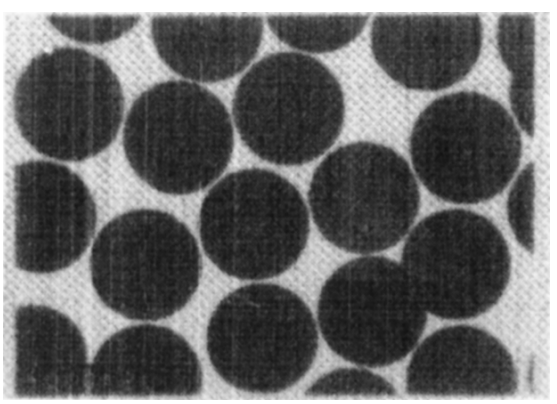

Fig. 1 Scanning electron micrograph of 2 μm non-porous silica beads

The amount of APS bonded was 56.5μmol/g from elementary analysis. The amount of dye immobilized on the modified silica was 2mg/ml by measuring the UV absorption at 620nm. This value was higher than 1.2mg/ml which was obtained by Unger[5].

Cibacron Blue F3GA leakage would interfere the assay of protein because of its UV absorption. The pH stability test showed that there was no significant dye leakage from pH3.0~6.0(Fig.2). In spite of the higher Cibacron Blue F3GA concentration at higher or lower pH value, the highest dye leakage was less than 25mg/L, which would not interfere the assay of protein.

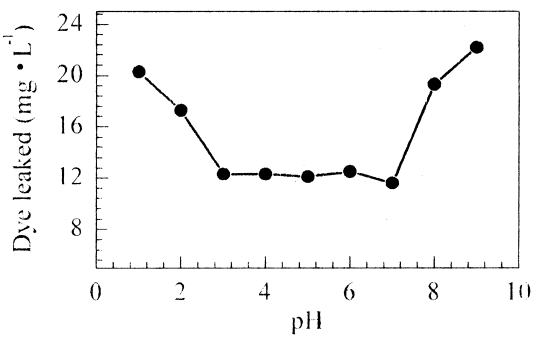

Fig. 2 pH stability test

Chromatographic Procedure

The interaction of protein and triazine dye is very complex. Ion exchange and hydrophobic interaction were expected owing to the presence of amino, sulphonic groups and polycylic aromatic rings in the dye molecule. We studied the effect of different experimental conditions on the chromatography of lysozyme. The adsorption of lysozyme increased with the pH value (Fig.3). Lysozyme is positive at pH 5.0~8.0 because of its high pI (11.0). In case of higher pH values, dye bears higher density of negative charges and ionic inter-

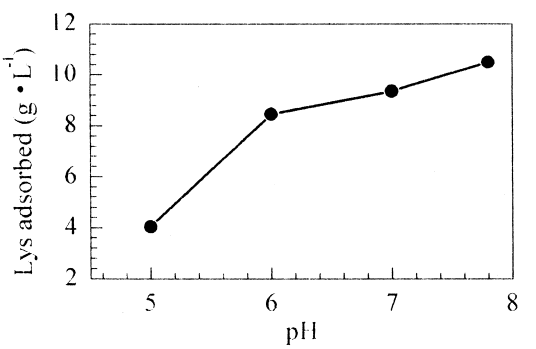

Fig. 3 The amount of lysozyme adsorbed at different pH value

action is enhanced. Fig.4 shows that the adsorption of lysozyme decreases with the increased of ionic strengths. It could be said that ion exchange was the dominant force between lysozyme and Cibacron Blue F3GA. So the elution could be carried out by increasing the ionic strength.

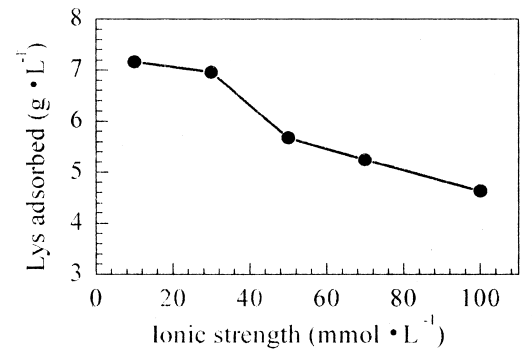

Fig. 4 The amount of lysozyme adsorbed at different ionic strength

Ovalbumin (Oval) and lysozyme from hen egg white showed different affinity to Cibacron Blue F3GA. Oval could not be retained. Its retention time did not vary with injection amount. Lysozyme was adsorbed on the column. Its mass concentration was proportional to the peak area(Fig.5). The mass recovery of lysozyme was more than 95%.

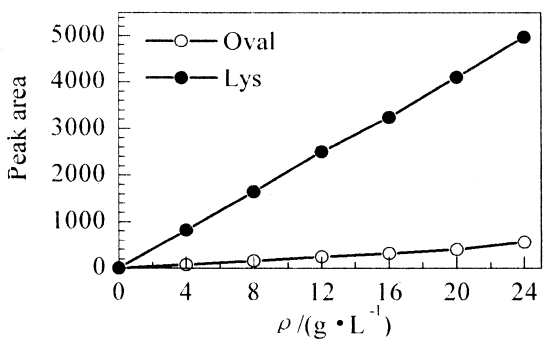

Fig. 5 Calibration graph of peak area vs concentration of oval and lysozyme in 10 μl sample.
Mobile phase: 50mmol/L Tris-HCl, pH7.8 (solution A); desorption solution: solution A containing 1mol/L KCl and volume fraction 20% glycol; flow rate: 0.6ml/min; detection: 280nm

Oval could not bind on the column alone but could bind with Zn^{2+} or Mg^{2+}[10]. So the column could be used to remove most of the oval in the when preparation of lysozyme from hen egg white. Fig.7 shows that most component of hen egg white does not bind to this packing and eluted in the breakthough peak. Lysozyme was bound and eluted by 1 mol/L KCl and 20% glycol (v/v). The purity of crude

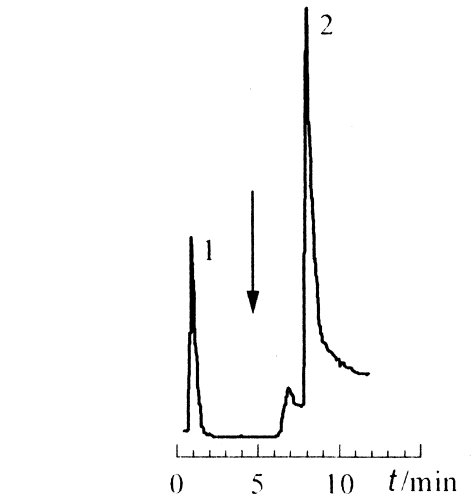

Fig. 6 Chromatogram of Oval and lysozyme
Chromatographic conditions as in Fig.5
1: Oval; 2: lysozyme

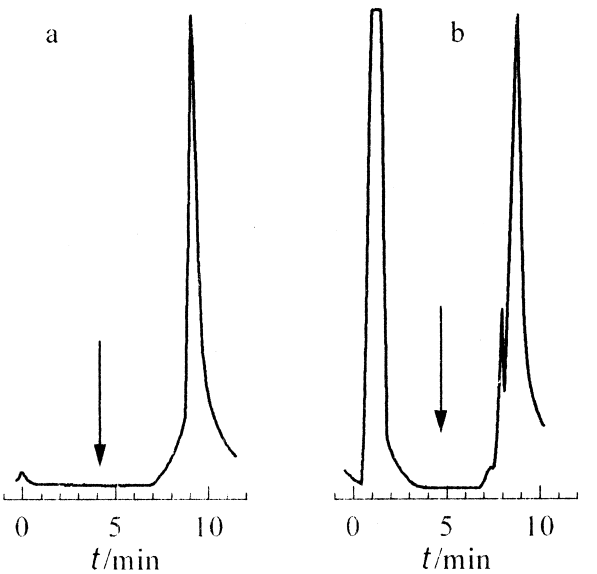

Fig. 7 Chromatogram of lysozyme and hen egg white on NPS-ACB column
Chromatographic conditions as in Fig.5
a: lysozyme; b; hen egg white

lysozyme was monitored using the non-porous silica-diethylamine(NPS-DEA) column. The retention time of the crude lysozyme with and without dialysis was accordant to that of lysozyme(Fig.8).

In addition, α-globulin could not be retained by the packing, while β- and γ-globulin could be adsorbed on the column. γ-Globulin could be eluted by 1 mol/L KCl and 20% glycol (v/v), while β-globulin was not able to be eluted by the same eluent(Fig.9). The difference in affinity interaction could be used to separate the three

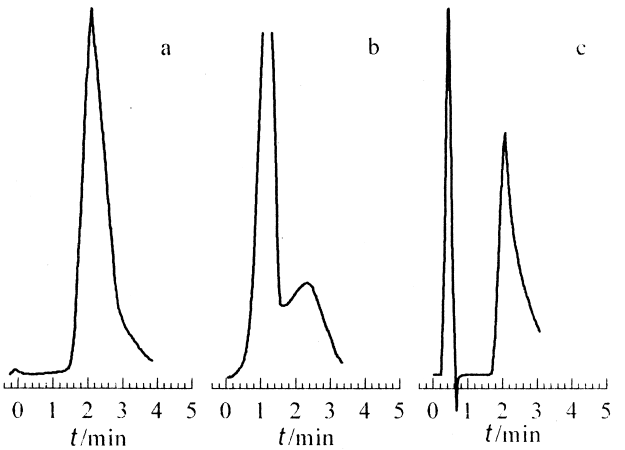

Fig. 8 Chromatogram of crude lysozyme on NPS-DEA column
Mobile phase: 0.5mol/L NaCl in 50mmol/L Tris-HCl (pH8.0, adjusted with HCl); flow rate: 0.7ml/min; detection: 280nm
a: lysozyme; b: crude lysozyme, without dialysis; c: crude lysozyme, with dialysis

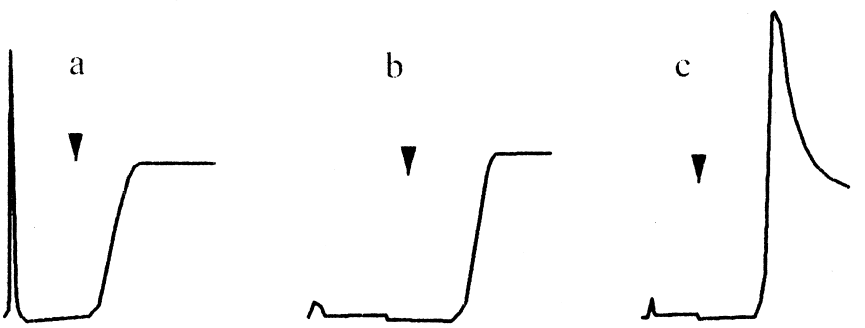

Fig. 9 Chromatogram of α, β, γ-globulin
Chromatographic conditions as in Fig.5
a: α-globulin; b: β-globulin; c: γ-globulin

globulins rapidly.

Conclusion

In this paper, non-porous silica was used as matrix, Cibacron Blue F3GA as ligand to attain an affinity packing NPS-ACB. The amount of ligand attached reached 2mg/ml. The stability test of pH indicated that this packing is stable. It could be used to preparation of lysozyme from hen egg white in a small scale and rapid separation of α-, β-, γ-globulin.

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