Thiophilic Interaction Chromatography of Serum Albumins

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Abstract

An investigation of the binding of native and recombinant human serum albumin and bovine serum albumin on three thiophilic gels, PyS, 2S, and 3S was performed. In addition to these proteins, we studied serum albumins from several species such as goat, rabbit, guinea pig, rat, hamster, baboon, and pig. Our results reveal that recombinant human serum albumin (rHSA) binds completely to PyS whereas native human serum albumin and bovine serum albumin bind only partially to PyS. The binding affinities of rHSA, human serum albumin and bovine serum albumin to 2S and 3S gels are less than their binding to PyS. Serum albumins from goat, rabbit, guinea pig, rat, hamster, baboon, and pig bind much stronger to 3S gel than human and bovine serum albumins. The binding of pig and hamster serum albumins is stronger than that of rat, goat, baboon, and rabbit.

Introduction

Thiophilic interaction chromatography (TIC) was introduced by Jerker Porath and coworkers (1,2). The possible amino acid "signatures" have been identified to be aromatic amino acids: tryptophan, phenylalanine, and tyrosine. In the analysis of the mechanism of thiophilic adsorption, it has been observed that single amino acids [(trp(W), phe(F), or tyr (Y)] did not bind to T-gels. However, $(Trp)_2$ >> $(Phe)_2$ >> $(Tyr)_2$ were bound. Apparently, it takes an aromatic cluster to generate a binding affinity for a T-gel (2). It has been shown that TIC is an effective method for studying the binding of proteins containing clusters of aromatic residues such as tryptophan, phenylalanine, and tyrosine (3,4). The topic of thiophilic adsorption has been reviewed recently (5,6), and an excellent review on the use of this technique for antibody purification has been published by Boschetti (7). Quite recently, TIC has been applied to purify several proteins, including the prostate specific antigen (PSA) at our institute (8,9). In an earlier communication from our laboratory, we reported the TIC of human serum transferrin and lactoferrin and its potential application to quantify the levels of these transferrins in serum of Alzheimer's patients (10). TIC of amyloid β-peptides of interest in Alzheimer's disease has also been reported from our laboratory (11). We have also reported the binding of mammalian and avian transferrins to the thiophilic gels, PyS, 2S, and 3S (12).

T-gels are made of an inert stationary phase (agarose) with different active sites, also known as ligands. These contain the sulfur lone pairs, which interact with the aromatic clusters on the surface of the protein (π-lone pair interaction). There are four known T-gels: PyS, 2S, 3S, and 4S (Figure 1). The name refers to the number of sulfur atoms in the ligand, which is a preferential site for binding of aromatic residues. The proteins

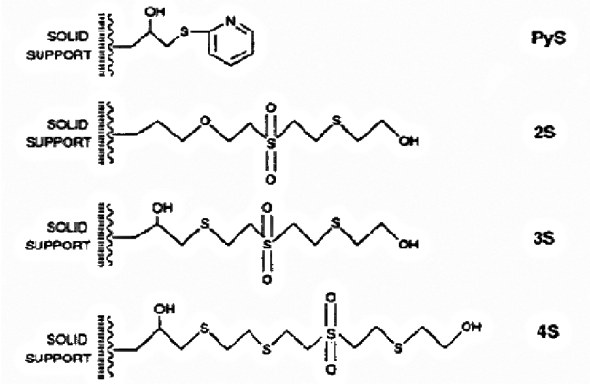

Figure 1. Structure of T-gels.

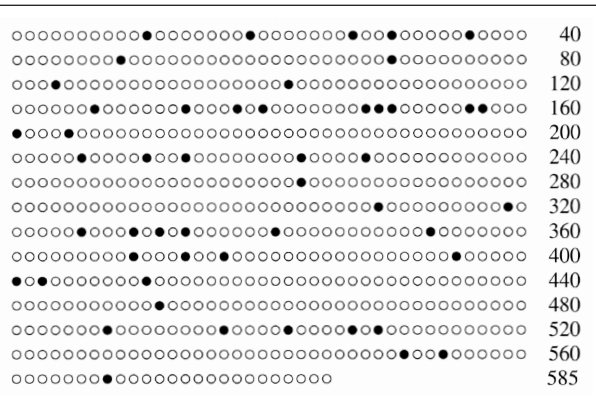

Figure 2. Amino acid sequence of human serum albumin with the aromatic amino acids represented as solid black dots.

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that can bind to T-gels must possess clusters of aromatic residues, which contain π electron clouds. An analysis of the primary sequence of human serum albumin (Figure 2) reveals the presence of several aromatic amino acids, which are hilghlighted in red. Also, the crystal structure of human serum albumin (13–15) revealed the presence of several of these amino acids on the surface of the bilobal proteins (Figure 3). This prompted an investigation of the binding of human and other mammalian serum albumins to T-gels. We report the details of our study in this publication.

Materials and Methods

The following components constituted the high-performance liquid chromatography (HPLC) setup used for TIC: Waters 600 multi-solvent delivery system (Waters Millipore, Milford, MA) from Millipore Corporation with 200 µL pump heads; rheodyne Model 7125 syringe loading sample injector with a 2 mL sample loop; Waters 994 programmable photodiode array detector; Waters 470 fluorescence detector; micro flow cell and pH microelectrode from Amersham Pharmacia Biotechnology (Piscataway, NJ); baseline 810 chromatography work station software from Millipore Corporation installed on an HP Vectra PC (Hewlett Packard, Palo Alto, CA).

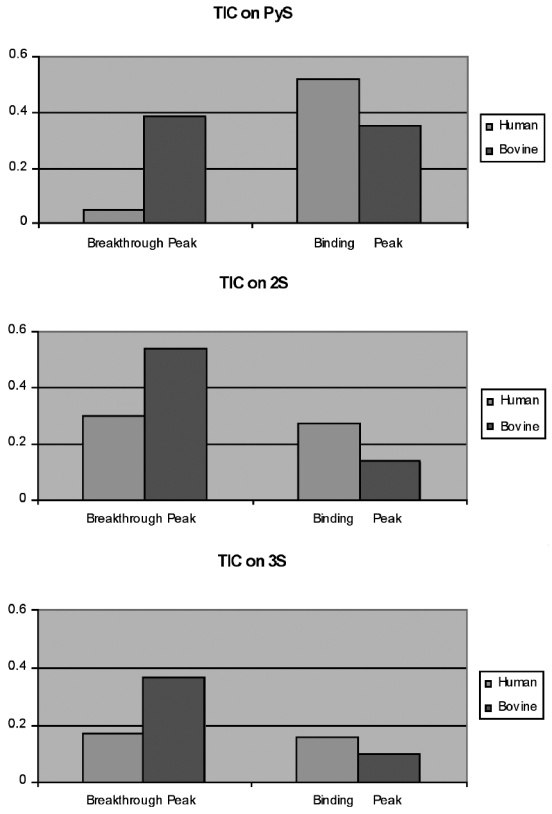

Figure 5. Relative absorbances of the breakthrough and binding fractions of HSA, and BSA on PyS, 2S, and 3S gels.

For TIC, the gradient program for albumin analysis included the following protocol: (0–10 min) 1M Na_2SO_4, 20mM PO_4 at pH 7.4 and a linear gradient from 10–40 min. The proteins were detected using the Waters 994 photodiode detector. The detector monitored absorbances from 200 nm to 400 nm. The absorbance readings at 280 nm were acquired using the software from Waters 470 fluorescence detector. The solvents used for the

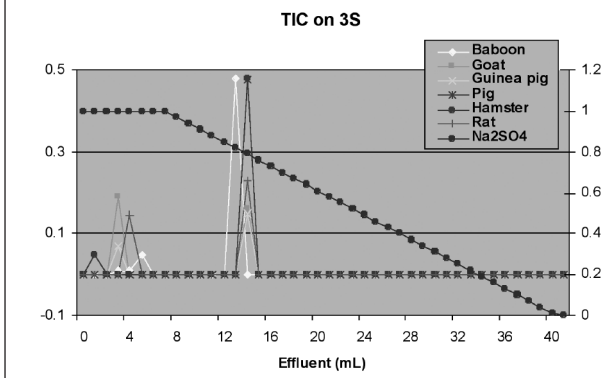

Figure 6. TIC of baboon, goat, guinea pig, hamster, and rat serum albumins on 3S gel.

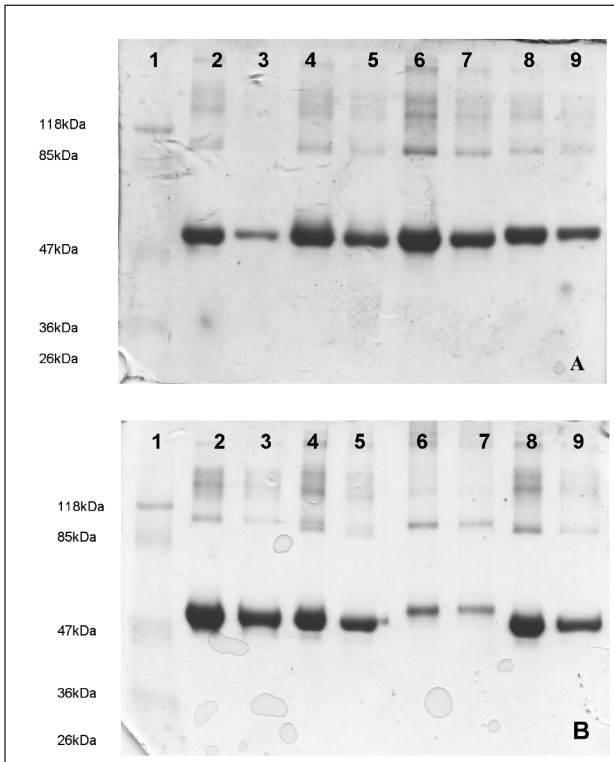

Figure 8. SDS-PAGE of serum albumins and their binding fractions. Lane: 1, Molecular Weight Marker (MWM); 2, BSA; 3, binding fraction of BSA; 4, pig serum albumin; 5, binding fraction of pig serum albumin; 6, rabbit serum albumin; 7, binding fraction of rabbit serum albumin; 8, rat serum albumin; binding fraction of rat serum albumin (A). Lane: 1, MWM; 2, native human serum albumin; 3, binding fraction of HSA; 4, goat serum albumin; 5, binding fraction of goat serum albumin; 6, guinea pig serum albumin; 7, binding fraction of guinea pig serum albumin; 8, baboon serum albumin; and 9, binding fraction of baboon serum albumin (B).

experiments were 1M Na_2SO_4, 20mM PO_4 at pH 7.4 and 20mM phosphate buffer, pH 7.4. The column was washed with 10 column volumes (30 mL) of starting solution (1M Na_2SO_4, 20mM PO4 at pH 7.4) before each experiment. The serum albumins bind to a T-gel at high concentration of lyotropic salt and are released as its level was decreased. The binding fractions were collected and the presence of albumin were confirmed by SDS-PAGE on a 12% polyacrylamide gel.

Human, bovine serum albumin, and albumin from goat, rabbit, guinea pig, rat, hamster, baboon, and pig were purchased from Sigma Aldrich Chemical Co. (Saint Louis, MO). Recombinant serum albumin was gift from Delta Biotechnology Inc., UK. The PyS and 2S gels were purchased from Sigma Aldrich chemical Corp. and the 3S gel was obtained from EM scientific company (Carson City, NV). All samples of serum albumins were prepared by dissolving 1 mg of commercially purchased protein in 1 mL of 1M Na_2SO_4, 20mM PO_4 at pH 7.4. Prior to the injection of the sample, the sample was centrifuged. An HR 10/2 column was purchased from Amersham Pharmacia biotechnology (Piscataway, NJ). The column was designed for use on a FPLC (fast performance liquid chromatography) system and appropriate FPLC/HPLC unions (P 626 10-32 adapter) were purchased from Upchurch scientific, Inc. This column was packed with PyS, 2S, and 3S thiophilic gels. All experiments were done at a constant flow rate of 0.5 mL/min at room temperature (20°C). All solvents were degassed and purged using helium gas as per the recommendation of the instrument supplier.

Results and Discussion

Figure 4 illustrates the TIC of rHSA, native human HSA, and bovine HSA on the three gels, PyS, 2S, and 3S. The rHSA binds completely to PyS but on 2S and 3S gels it binds less. The binding of native human HSA is much less than rHSA on PyS and also on 2S and 3S gels. The binding of BSA is very similar to HSA. Figure 5 illustrates the relative absorbances of the breakthrough and binding fractions of rHSA, HSA, and BSA on PyS, 2S, and 3S gels. We then investigated the binding of serum albumin from goat, rabbit, guinea pig, rat, hamster, baboon, and pig on the three T-gels (Figure 6) All these serum albumins bound much stronger than the bovine or human albumins on 3S gel. Pig and hamster serum albumin bound much stronger than the other albumins to 3S gels. Figure 7 illustrates the relative absorbances of the break through and the binding fractions of albumin from goat, rabbit, guinea pig, rat, hamster, baboon, and pig serum albumins on 3S gels. Figure 8 gives SDS- PAGE of the different serum albumins (human, bovine, pig, rabbit, goat, rat, hamster, baboon, and guinea pig). In each case, the binding fraction is purer than the original sample.

Conclusion

The binding or non binding of albumins to thiophilic gels out of sera has been somewhat controversial (1–3). It has been recently reported that the binding of albumins to thiophilic gels is impaired by the competition with other proteins present in the sera (5,6). Our study with purified albumins confirms this conclusion.

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