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 HSA, 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

An abstract is not provided.
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 highlighted 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 micro-electrode 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).

For TIC, the gradient program for albumin analysis included the following protocol: (0–10 min) 1M Na₂SO₄, 20mM PO₄ 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...
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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