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Binding of 6-propyl-2-thiouracil to human serum albumin destabilized by chemical denaturants

J. Równicka-Zubik [a], A. Sułkowska [a,*], B. Bojko [a], M. Maciążek-Jurczyk [a], J. Pożycka [a], D. Pentak [b], W.W. Sułkowski [b]

[a] Department of Physical Pharmacy, Medical University of Silesia, Jagiellońska 4, 41-200 Sosnowiec, Poland
[b] Department of Environmental Chemistry and Technology, Institute of Chemistry, University of Silesia, Szkolna 9, 40-006 Katowice, Poland

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ABSTRACT

We compared the binding affinity of 6-propyl-2-thiouracil (PTU) with native and destabilized human serum albumin (HSA) as a model to assess the binding ability of albumin in patients suffering from chronic liver or renal diseases. Urea (U) and guanidine hydrochloride (Gu·HCl) at a concentration of 3.0 M were used as denaturation agents.

Increasing the concentration of PTU from 0.8×10^{-5} to 1.20×10^{-4} M in the systems with HSA causes a decrease in fluorescence intensity of the protein excited with both 280 and 295 nm wavelengths. The results indicate that urea and Gu·HCl bind to the carbonyl group and then to the NH-group. To determine binding constants we used the Scatchard plots. The presence of two classes of HSA–PTU binding sites was observed. The binding constants (K_b) are equal to $1.99 \times 10^4\ M^{-1}$ and $1.50 \times 10^4\ M^{-1}$ at $\lambda_{ex} = 280$ nm, $5.20 \times 10^4\ M^{-1}$ and $1.65 \times 10^4\ M^{-1}$ at $\lambda_{ex} = 295$ nm. At $\lambda_{ex} = 280$ nm the number of drug molecules per protein molecule is $a_I = 1.45$ and $a_{II} = 1.32$ for I and II binding sites, respectively. At $\lambda_{ex} = 295$ nm they are $a_I = 0.63$ and $a_{II} = 1.54$ for the I and II binding sites.

The estimation of the binding ability of changed albumin in the uremic and diabetic patients suffering from chronic liver or renal diseases is very important for safety and effective therapy.

1. Introduction

Mammalian albumin is a widely studied protein synthesized by the liver as preproalbumin. The majority of the human serum albumin (HSA) can be found in blood plasma in concentrations ranging from 40 to 50 g/l. The HSA molecule contains 585 amino acid residues that form a polypeptide chain. Based on crystallographic studies it is known that the albumin molecule is heart shaped with dimensions $\sim 8 \times 3$ nm [1]. This molecule plays an important role in the human body because it is the main carrier protein for substances which have no specific transporters (e.g. fatty acids), but it also transports other substances that have their own specific carriers. Albumin also behaves as a buffer because it is capable of binding to acids and basis. Albumins also function as a protein reserve when the organism is deficient in protein.

2-*n*-propyl-6-thiouracil (PTU) is an antithyroid drug widely used to inhibit thyroid hormone synthesis in humans and animals. PTU interferes with thyroid peroxidase – mediated iodination of tyrosine residues in thyroglobulin, an important step in the synthesis of thyroxine and triiodothyronine. PTU can block the conversion of thyroxine to triiodothyronine within the thyroid and in peripheral tissues [2].

Denaturation is a conformational disorder of the polypeptide chain which results in the alteration of the protein tertiary structure. Denaturing agents destroy hydrogen bonds without disrupting covalent bonds. Urea and guanidine hydrochloride are common denaturation agents that cause conformational changes in the native protein and are often used to obtain denaturated (unfolded) states [3,4]. It has been previously shown that urea and guanidine hydrochloride can bind to protein peptide bonds [5]. In the unfolded protein more peptide groups are exposed to denaturant molecules. It has been shown that the mechanism of denaturing activity for urea and guanidine hydrochloride are completely different although both lead to the unfolding state [6–8]. The differences in the interaction of the urea and guanidine hydrochloride action appear to be based on the ionic property of Gu·HCl and its absence in urea [6,9]. At high concentrations both urea and Gu·HCl result in a loss of tertiary structure of albumin [10–13].

Urea at concentration of 3 M and 3 M Gu·HCl induce partial unfolding in albumin, which is known as the "molten globule" state. Modification of the albumin tertiary structure causes changes in the mechanism of the ligand binding. It is hypothesized that the binding of drugs to albumin in the pathological state are diminished. In oncological patients a modification of the N-termi-

* Corresponding author. Tel.: +48 32 3641582.
E-mail address: asulkowska@sum.edu.pl (A. Sułkowska).

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nal amino acids, 50% of tyrosyl groups, free SH-group of cysteine and lysine group in the fourth position in the N-terminal sequence of amino acids have been observed [14]. Troitskii et al. [15] compared albumin from blood serum of healthy individuals with those of patients with various pathologies using isoelectric point focusing in a borate-polyol system. By using an optical rotatory dispersion technique they found that the modified protein corresponding immunochemically to the HSA did not contain polymers, but had a lower content of α-helix structure than the native one. Alterations within the tertiary structure of albumin were observed in patients suffering in chronic renal and liver diseases. Ivanov et al. [16] showed dramatic structural changes of cirrhotic serum albumin affecting its secondary structure. Modification of albumin structure caused by oxidation has been detected in the blood plasma of patients with diabetes and kidney disease [17,18].

The aim of this study is to obtain in vitro albumin in a state similar to the pathological one by chemical destabilization and to compare the binding affinity of PTU with native and destabilized albumin.

2. Materials and methods

2.1. Chemicals

Crystallized and lyophilized human serum albumin (HSA), fraction V were purchased from ICN Biomedicals, Inc. (USA), guanidine hydrochloride (Gu·HCl) and urea (U) from ICN Biochemicals, Inc. (USA), 6-*n*-propyl-2-thiouracil (2-thio-4-hydroxy-6-*n*-propylpyrimidine, PTU) obtained from Sigma–Aldrich Chemie, Gmbh P.O. (Germany).

2.2. Fluorescence measurement

Measurements were performed on a Kontron SFM Instrument AG spectrofluorimeter with $1 \times 1 \times 1\ cm^3$ cells and slits of 5/10 nm. The fluorescent emission spectra were recorded at room temperature at two excitation wavelengths: $\lambda_{ex} = 280$ nm and $\lambda_{ex} = 295$ nm and they were displayed in terms of relative fluores-

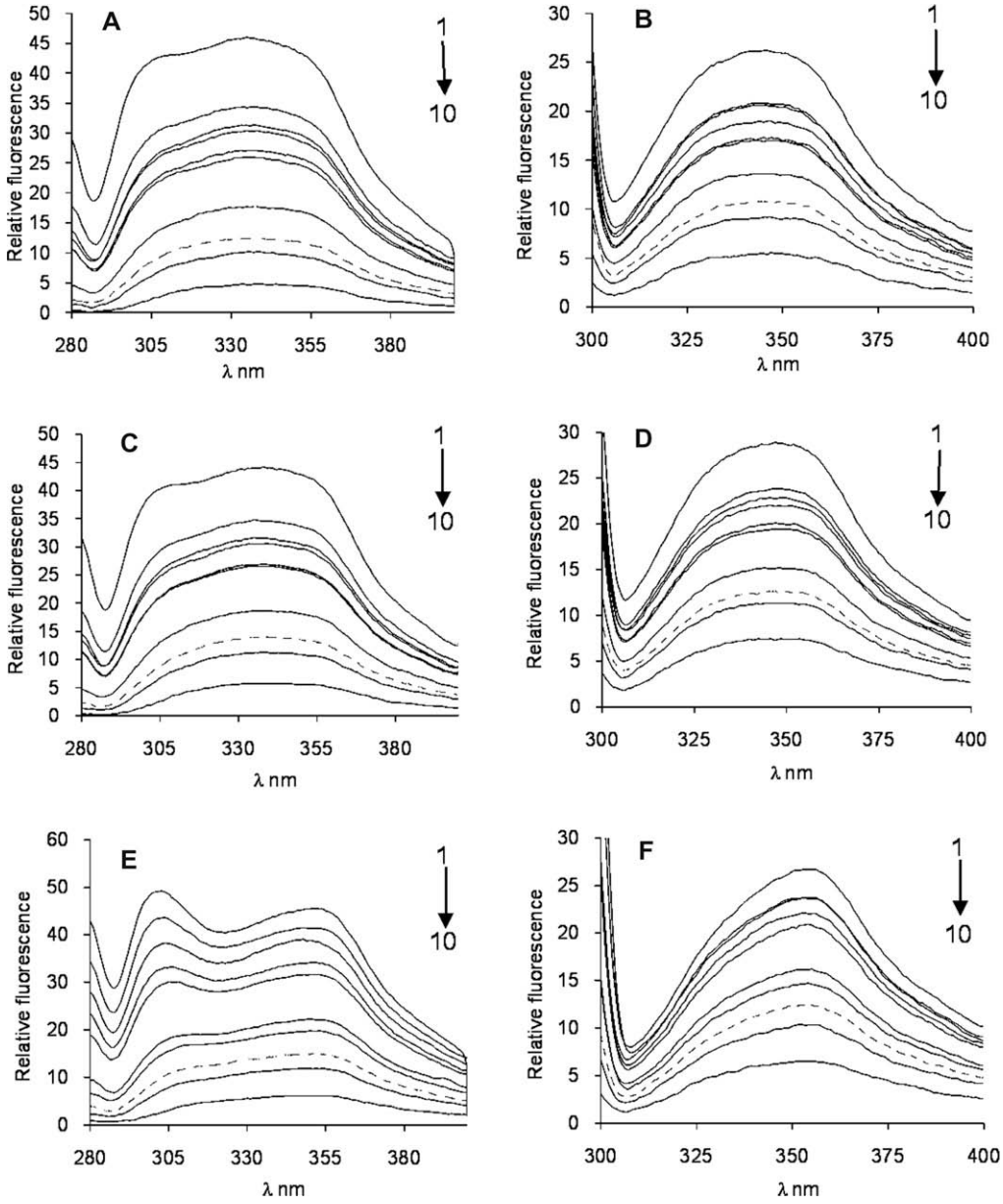

Fig. 1. The fluorescent emission spectra of (1) HSA (8×10^{-6} M) in water (A, B), in 3 M urea (C, D), in 3 M guanidine hydrochloride (E, F). PTU concentration: (2) 0.8×10^{-5} M (3) 1.6×10^{-5} M (4) 2.4×10^{-5} M (5) 3.2×10^{-5} M (6) 4.0×10^{-5} M (7) 4.8×10^{-5} M (8) 6.4×10^{-5} M (9) 8.0×10^{-5} M (10) 12.0×10^{-5} M. Spectra (8) are drawn with dashed lines. The fluorescence excitation wavelengths: 280 nm (A, C, E) and 295 nm (B, D, F).