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Comparative studies on drug binding to the purified and pharmaceutical-grade human serum albumins: Bridging between basic research and clinical applications of albumin

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ABSTRACT

Human serum albumin (HSA), the most abundant protein in blood plasma, is a monomeric multidomain protein that possesses an extraordinary capacity for binding, so that serves as a circulating depot for endogenous and exogenous compounds. During the heat sterilization process, the structure of pharmaceutical-grade HSA may change and some of its activities may be lost. In this study, to provide deeper insight on this issue, we investigated drug-binding and some physicochemical properties of purified albumin (PA) and pharmaceutical-grade albumin (PGA) using two known drugs (indomethacin and ibuprofen). PGA displayed significantly lower drug binding capacity compared to PA. Analysis of the quenching and thermodynamic parameters indicated that intermolecular interactions between the drugs and the proteins are different from each other. Surface hydrophobicity as well as the stability of PGA decreased compared to PA, also surface hydrophobicity of PA and PGA increased upon drugs binding. Also, kinetic analysis of pseudo-esterase activities indicated that K_m and V_{max} parameters for PGA enzymatic activity are more and less than those of PA, respectively. This *in vitro* study demonstrates that the specific drug binding of PGA is significantly reduced. Such studies can act as connecting bridge between basic research discoveries and clinical applications.

1. Introduction

The name "albumin" originates from the Latin word "albus" (white) or "albumen" (whiteness) [1]. Albumin is the most abundant plasma protein, with a normal concentration in the range of 35–45 g/L. Structurally; it comprises a single peptide chain with 585 amino acids held in three homologous domains by 17 disulfide bonds, forming a heart-shaped molecule (Fig. 1A) [2,3]. Albumin which is produced in the liver has several physiological functions including: contributing to the colloid osmotic pressure and facilitates the transport, distribution, and metabolism of many endogenous substances, such as fatty acids, amino acids, bile acids/salts, and hormones, and exogenous substances such as drugs [4]. Moreover, for many hormones and vitamins, albumin acts not only as a carrier, but also as a reservoir and for a number of endogenous and exogenous toxins are sequestered by albumin and, in that way, rendered harmless in the circulation.

Human albumin solutions (pharmaceutical-grade albumins) were developed in the United States during the Second World War [5]. The traditional method for its isolation, first described by Cohn and colleagues in 1946 [6]. Some manufacturers employed chromatographic processing of plasma, a process that provides both a purer product and a higher yield. However, during the heat sterilization process [7], oxidative quality and some of physiological activities and hence the drug binding characteristics of the protein may changed [8,9].

The drug-binding properties of human serum albumin (HSA) have been extensively studied. The binding of drugs to plasma

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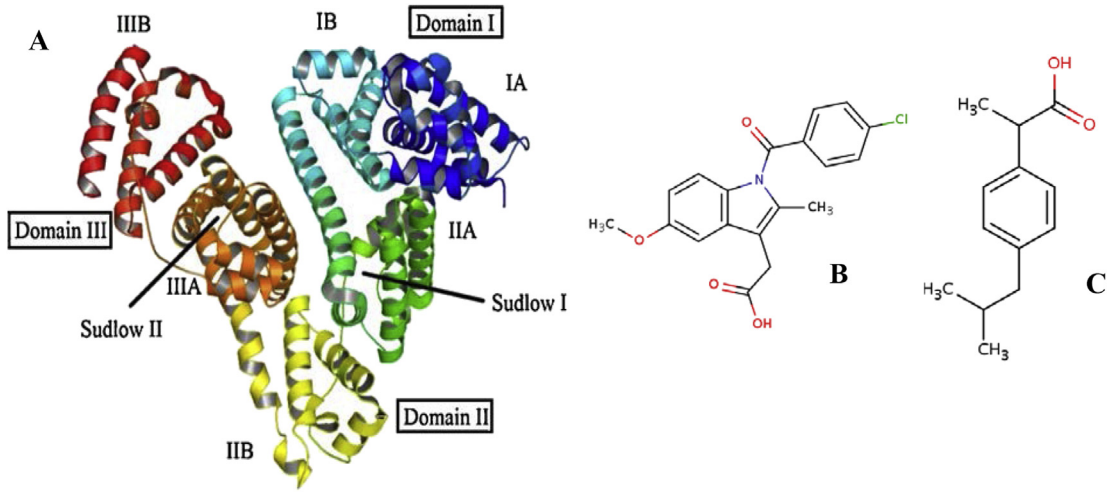

Fig. 1. (A) Crystal structure of HSA and the locations of its domains as well as drug binding sites. The positions of Sudlow's site I, site II and each domain are indicated. The crystal structure of protein was obtained from Protein Data Bank (ID code: 2BXB). Chemical structures of (B) indomethacin and (C) ibuprofen.

proteins (especially albumin) affects their distribution and rate of metabolism [10]. Crystal structure analyses have revealed that HSA has two hydrophobic pockets as binding sites for aromatic and heterocyclic ligands; one site in subdomain IIA (commonly referred to as Sudlow's site I: warfarin-binding site) and the other within subdomain IIIA (commonly referred to as Sudlow's site II: indole/benzodiazepine site) [11]. Both hydrophobic and electrostatic interactions play a major role in controlling the affinity towards drug binding for sites I and II; for site I, mainly hydrophobic interactions are dominant, whereas for site II, a combination of hydrophobic, hydrogen bonding, and electrostatic interactions all play a crucial role [11,12].

Indomethacin and ibuprofen were selected as test drugs. According to literature [13–15], both drugs represent different binding sites on the albumin molecule including drug binding sites on the domains IB, IIA, IIA–IIB and/or IIIA (see Fig. 2). Indomethacin (Fig. 1b) (2-{1-[(4-chlorophenyl) carbonyl]-5-methoxy-2-methyl-1H-indol-3-yl} acetic acid) is a non-steroidal anti-inflammatory drug (NSAID) commonly used as a prescription medication to reduce fever, pain, stiffness, and swelling [16]. Ibuprofen (Fig. 1c) ((RS)-2-(4-(2-methylpropyl) phenyl) propanoic acid) also is a nonsteroidal anti-inflammatory drug used for relieving pain and reducing inflammation [16]. In this study we investigated/compared binding properties (such as binding constant, number of binding sites and forces involved in the protein–drug interaction) of the drugs; ibuprofen and indomethacin to PA and PGA by various spectroscopic methods such as intrinsic and extrinsic fluorescence and UV–vis spectroscopy. Also, contrary to previous report [17], we set out to determine whether surface hydrophobicity, thermodynamic stability, and psudo-esterase activity of PGA differs from the native albumin (PA) or not.

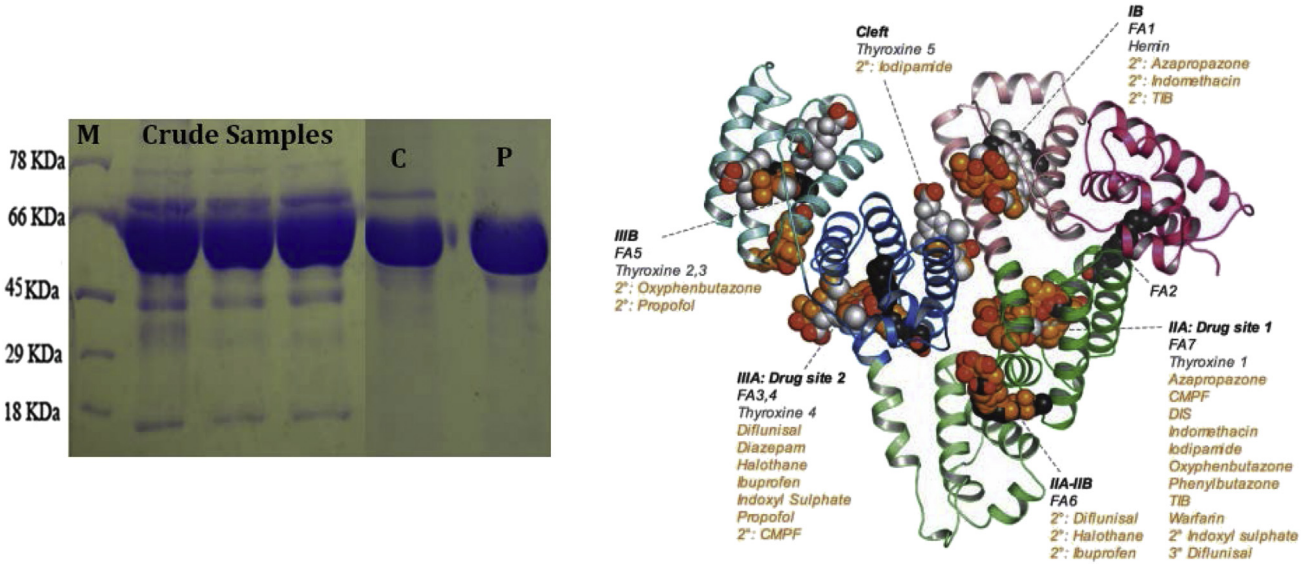

Fig. 2. (Left) SDS-PAGE pattern of the crude samples, purified albumin (lane P), compared to the commercial protein (lane C), M; molecular weight markers. (Right) Summary of the ligand binding capacity of HSA as defined by crystallographic studies. Ligands are depicted in space-filling representation (taken from Ref. [14]).

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