Biochimica et Biophysica Acta xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Structural studies of several clinically important oncology drugs in complex with human serum albumin ☆

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ARTICLE INFO

Article history:
Received 7 May 2013
Received in revised form 25 June 2013
Accepted 26 June 2013
Available online xxxx

Keywords:
Human serum albumin
Structure
Oncology
Camptothecin
9-amino camptothecin
Etoposide
Teniposide
Bicalutamide
Idarubicin
Drug design

ABSTRACT

Background: Serum albumin is a major pharmacokinetic effector of drugs. To gain further insight into albumin binding chemistry, the crystal structures of six oncology agents were determined in complex with human serum albumin at resolutions of 2.8 to 2.0 Å: camptothecin, 9-amino-camptothecin, etoposide, teniposide, bicalutamide and idarubicin.
Methods: Protein crystal growth and low temperature X-ray crystallography
Results: These large, complex drugs are all bound within the subdomain IB binding region which can be described as a hydrophobic groove formed by α-helices h7, h8 and h9 covered by the extended polypeptide L1. L1 creates a binding cavity with two access sites, one between loop L1 and α-helices h7 and h8 (distal site: IB_d) and the other between L1 and α-helix h9 (proximal site: IB_p). Camptothecin (2.4 Å) and 9 amino camptothecin (2.0 Å) are clearly bound as the open lactone form (IB_p). Idarubicin (2.8 Å) binds in a DNA like dimer complex via an intermolecular π stacking arrangement in IB_d. Bicalutamide (2.4 Å) is bound in a folded intramolecular π stacking arrangement between two aromatic rings in IB_d similar to idarubicin. Teniposide (2.7 Å) and etoposide (2.7 Å), despite small chemical differences, are bound in two distinctly different sites at or near IB. Teniposide is internalized via primarily hydrophobic interactions and spans through both openings ($IB_{p\text{-}d}$). Etoposide is bound between the exterior of IB and IIA and exhibits an extensive hydrogen bonding network.
Conclusions: Subdomain IB is a major binding site for complex heterocyclic molecules.
General significance: The structures have important implications for drug design and development. This article is part of a Special Issue entitled Serum Albumin.

1. Introduction

Serum albumin, produced in abundance by the liver, is the major protein of the circulatory and lymphatic system. There it contributes to many physiologically important functions including colloidal oncotic blood pressure (80%) and to the maintenance of blood pH. It is also the principal transport protein where its prolific binding properties facilitate the transfer and distribution of essential vitamins and nutrients, and provide protection through the sequestration of toxic metabolites. The reader is referred to the lucid review and compilation by Peters of these and many other properties of albumin [1]. Owing to its importance as a circulatory protein, albumin shares an unusually long half-life of 18 days with the immunoglobulins, both of which result from a similar, but distinct active FcRn dependent process [2].

Structurally, albumin is a 585 amino acid protein, which is the product of three tandem gene duplications. These three gene domains are homologous (I, II, III) and coalesce to form a predominantly alpha-helical heart-shaped molecule (Fig. 1), highly cross-linked by 17 disulfides. Each domain in turn is comprised of two subdomains, denoted A and B [3,4].

One of the interesting properties of albumin is the molecule's ability to reversibly bind and transport a plethora of small hydrophobic and anionic molecules, a property for which Peters has colorfully described albumin as the "tramp steamer" of the circulatory system [1]. Albumin's prolific binding properties have been extensively studied from the earliest days of albumin research. These transported molecules in addition to endogenous ligands, include to a lessor or greater degree, the entire pharmacopeia. Its ligand binding also imparts a protective function through the sequestration of metabolites, such as heme and its toxic

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0304-4165/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.bbagen.2013.06.032

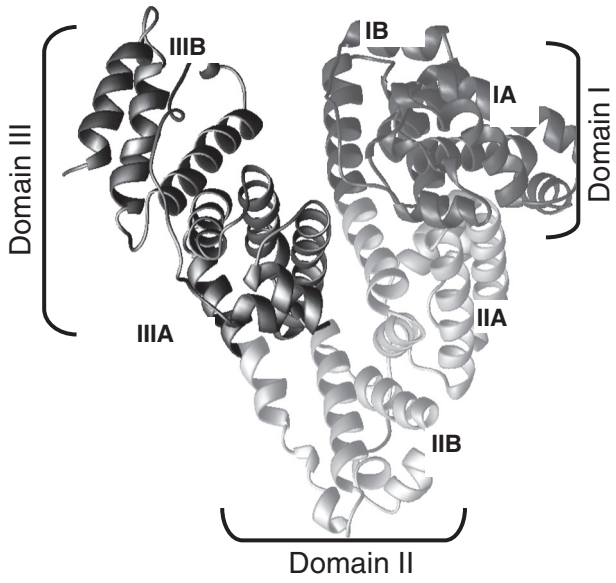

Fig. 1. A ribbon diagram illustrating the overall topology of human serum albumin and its domain and subdomain structure. Reproduced by permission from the author and publisher John Wiley & Sons.

metabolite, bilirubin [1,5]. It is undoubtedly this protective function that belies its importance as a major pharmacokinetic effector of pharmaceuticals. Early structural studies revealed the basic chemical nature of these binding attributes [3,4] and together with more recent studies, including this work, a greater understanding of albumin binding chemistry continues to emerge [6,7].

Pharmaceutical applications of albumin are numerous and include its extensive use as a volume expander of choice, as an excipient and stabilizer in vaccines and other biologics, and for stem cell differentiation and other cell culture methods used in the production of pharmaceuticals (such as antibody production in CHO cells) [1]. In recent years, there has been a growing number of biologics in clinical trials, which have sought to take advantage the unusually long plasma half-life of albumin as a means to extend therapeutic half-life. Approaches to circumvent undesirable albumin interactions have included pegylation of small molecules and liposomal encapsulation [8,9]. While many approaches have sought to extend drug half-life by creating covalent adducts with albumin or in the case of biologics, albumin fusion proteins, see for example the recent work of Martinowitz and Lubetsky [10]. The approach taken here aims to understand the albumin binding interaction with pharmaceuticals in atomic detail and ideally, to use this information together with knowledge of the drug target to design improvements, such as, lowering the effective dose and/or improving the efficacy of the target drug. To advance the understanding of the specific nature of human serum albumin drug transport for the purposes of drug design and development, we undertook a large scale crystallographic survey which produced structures of albumin complexes with a broad spectrum of pharmaceuticals and important endogenous ligands [6]. To accomplish this goal, a number of challenging obstacles to the survey had to be overcome. Experimental objectives of the research program included: (i) banking and qualifying thousands of chemical entities of potential scientific or medicinal interest; (ii) developing reproducible/robust co-crystallization methods to produce high quality crystals; and (iii) the establishment of cryogenic conditions for the storage and archival of the crystals as a prerequisite for data collection, especially at synchrotron facilities. Using the general approaches outlined herein, over 230 atomic structures of albumin in complex with various pharmaceuticals and endogenous ligands were determined, and included representative drugs in virtually every therapeutic category [6]. These studies confirmed the previously determined dominance of subdomains IIA and IIIA for small hydrophobic and anionic drugs, but surprisingly revealed that subdomain IB, at least for the selected group of ligands studied, was slightly favored over the other two sites, showing a high affinity and conformational flexibility to accomplish the binding of complex heterocyclic endogenous ligands and drugs. Indeed, there were early indications of specialized binding chemistry in domain I with solution studies of the recombinant domains [11,12], but there was some concern whether these observations were produced by hydrophobic exposure of surfaces normally occluded in the full length structure. These solutions studies and the high-resolution structure of hemalbumin corroborated the specialized binding site for heme in subdomain IB [13]. Because of the complexity of the binding site locations in total and to facilitate future discussions, rather than continue with binding site nomenclature based on Sudlow et al. [14], we have moved to the less ambiguous, more descriptive subdomain nomenclature (Fig. 2).

Albumin binding can be a major pharmacokinetic determinant for many drugs. In the case of many cytotoxic drugs, high affinity translates to higher dosing and potentially higher secondary toxicity. Knowledge of thechemistry and location of the binding interaction can be used to guide drug design efforts to improve the safety and efficacy. Here we present the structures and binding chemistry of six important oncology drugs: camptothecin, 9-amino-camptothecin, etoposide, teniposide, bicalutamide and idarubicin (see Table 1). Crystal structures for many of the targets for the aforementioned drugs are available, including in some cases, key metabolic enzymatic drug protein complexes, such as cytochrome P450 [15]. Taken together with SAR data, the availability of a more complete understanding of drug–protein transport and metabolic interactions, should provide improved guidance in modern structure-guided drug design and development leading to improvements in both new and existing pharmaceuticals.

2. Materials and methods

2.1. Materials

Plasma derived human albumin was purchased from Serologicals (Norcross, GA). PEGs were produced by Fluka Biochemika and purchased through Sigma Aldrich (St. Louis, MO). All other general laboratory chemicals were purchased from Sigma-Aldrich Chemical Company. CryoCap Copper™ pins and CryoLoops™ used for low temperature data

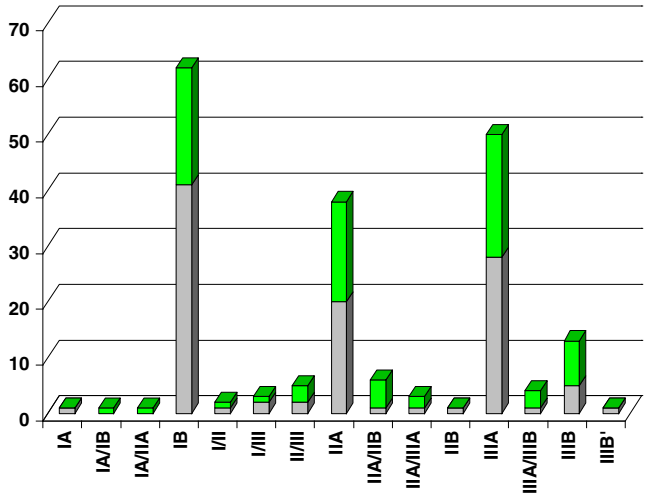

Fig. 2. Histogram of drug binding location and frequency for an early subset of drugs produced by the larger crystallographic survey [6], Green: number of total observations, Grey: Single site binders. Reproduced by permission from the author and publisher John Wiley & Sons.