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THREE-DIMENSIONAL STRUCTURE OF HUMAN SERUM ALBUMIN

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ABSTRACT

The binding locations to human serum albumin (HSA) of several drug molecules have been determined at low resolution using crystallographic methods. The principal binding sites are located within subdomains IIA and IIIA. Preliminary studies suggest that an approach to increasing the **in vivo** efficacy of drugs which are rendered less effective or ineffective by virtue of their interaction with HSA, would be the use of competitive displacement in drug therapies and/or the development of a general inhibitor to the site within subdomain IIIA. These findings also suggest that the facilitated transfer of various ligands across organ/circulatory interfaces such as the liver, kidney, and brain may be associated with binding to the IIIA subdomain.

Although serum albumin plays several important roles as the major protein of the circulatory system, it has been suggested that the molecule's high affinity for low molecular weight ligands may be its principal function (1). Over the past several decades there have been voluminous works describing the binding affinities and predicted locations of various endogenous and exogenous ligands to HSA. The reader is referred to the informative reviews by Kragh-Hannsen (2), Fehske et. al. (3), and Peters (4) and references cited therein. Previously, we described the structure of HSA as being comprised of three domains each of which were found to be the product of two helical subdomains consistent with the current understanding of the molecule's primary structure (5,6). These six subdomains have since been referred to as IA, IB, IIA, IIB, etc. We also reported that the binding of a chemically diverse group of compounds occurs primarily within two of these helical subdomains. These findings are in further agreement with several studies which indicate the presence of two to three high affinity binding sites within the molecule (2). More extensive experiments have recently been conducted by our group in an effort to identify the classical binding sites described by Sudlow and others (4,7). Sudlow grouped drug binding to HSA into three distinct categories, which he denoted as Site I for warfarin, Site II for the diazapines, and Site III for digitoxin. Although there is no complete agreement within the literature regarding these sites, warfarin is believed to bind primarily in domain II (3,4,8), the diazapines in domain III (9), and digitoxin, in an independent but unknown location (3,4). other studies indicate, however, that digitoxin competes with salicylates and warfarin for a common binding site (2,10). In this paper we present our preliminary findings regarding the major binding locations on HSA for several pharmaceutical compounds.

At the current resolution of 4.0 **A** the primary binding pockets within IIA and IIIA are cavities of approximately 8 **A** X 12 **A** X 18 **A** which are exposed to the solvent channels through an opening of -9-10 **A** in diameter. Large, well-ordered side chains protrude into these binding pockets where they appear to play a major role in ligand binding through stacking interactions. The identity of these amino acid residues should become evident once the model is fit to the electron density at higher resolution.

The general binding locations of several exogenous and endogenous ligands have been determined at various low resolutions using standard crystallographic methods, table 1. An illustration of various binding locations on HSA for several ligands is shown pictorially in figure 1. There exists, based on the current binding

studies, at least four distinct major binding regions on HSA. Location 1, subdomain IIIA, which is the most active and accommodating on HSA with few exceptions, e.g. warfarin, displays high affinities for most organic compounds. Location 2, subdomain IIA, has similar binding properties to IIIA but in these preliminary studies shows an absence of long-chain fatty acid binding. Based on the amino acids implicated, IIA may be inferred to be the bilirubin high affinity site. Location 3, the IA-IB interface is an additional binding site for aspirin (although weak), decanol, and possibly the short chain fatty acids. Location 4, subdomain IB, possesses a high affinity surface, thus far unique to long chain fatty acids and tryptophan. The general binding locations of the compounds of Sudlow's group, warfarin (Site I) , diazapam (Site II), and digitoxin (Site III), were determined crystallographically. The crystallographic studies are in general agreement with the expected locations indicating major binding sites for warfarin within IIA, and for diazapam in IIIA. Digitoxin, also shows a major binding site within subdomain IIIA and a secondary site at the interface between IA and IIA. Sudlows Site III then, differs from Site II only in the nature of the secondary binding sites.

It is interesting to note that the amino acid sequences most highly conserved between bovine, human , and rat albumins; Cys-34, Trp-212, sequence 143 through 155 and sequence 244 through 263 (11), all appear to be in regions involved in ligand binding. For example, residues 143 through 155, an aromatic sequence, corresponds with the region of Location 4, the second major long-chain fatty acid binding site. Certainly the correlation of the conserved amino acids sequences with occurrence of binding sites within HSA supports the idea that the principal role of this molecule in the circulatory system is to aid in the transport, distribution, and metabolism of both essential and foreign small molecules. Given the unique structure of serum albumin and the following interesting observations: (i) there is an asymmetric charge distribution within the primary structure of HSA at pH 7, of -9, -8, and -2 for domains I, II, and III respectively, thus indicating a potential amphoteric nature, (ii) HSA has been shown to interact with the cell membrane thereby aiding in the delivery of certain molecules, e.g. thyroxine, to the cell (12), (iii) subdomain IIIA appears to be the primary binding site for a variety of molecules, (iv) serum albumin is implicated in the facilitated transfer of ligands across various organ/circulatory interfaces, such as the liver, intestine , kidney, and brain; it is tempting to speculate whether the serum albumins possess membrane spanning properties. If this were the case, one might expect that HSA would unfold at the hinge region between IIA and IIB allowing the more lipophylic tail (subdomains IIB, IIIA, and IIIB) of HSA to span the membrane while the more polar head of the molecule (subdomains IA, IB, IIA) would remain on the surface of the membrane, thus releasing the bound ligands in subdomain IIIA into the cell. Perhaps then, ligand binding within subdomain IIIA is a prerequisite for the facilitated transfer of many endogenous and exogenous compounds at organ/circulatory interfaces.

As we continue to characterize the nature of bound ligands to serum albumin and correlate this information with a wealth of drug and ligand binding data in the literature; it will become possible to predict important displacement interactions for a variety of endogenous and exogenous ligands. Accordingly, this suggests that an approach to increasing the **in vivo** efficacy of drugs which are rendered less effective or ineffective by virtue of their interaction with HSA would be the use of competitive displacement in drug therapies and/or the development of a general inhibitor to the site within subdomain IIIA.

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TABLE I LIGAND BINDING LOCATIONS TO HSA

Data Set	Ligand	D	N	Rf	Obsv. Loc.	Pred. Loc.
HTRP1A	Tryptophan	5.0	3198	0.093	IIIA,IB,	IIA^IB
HPAL1A	Palmitate	5.9	2432	0.066	IIIA,IB	
HMYR1A	Myristate	6.0	1923	0.095	IIIA	
HMRGL1A	Glucose	5.8	3106	0.10	IIIA	
HSAA1A	Aspirin	4.0	7362	0.11	IIIA,IIA	
HMRWN1A	Warfarin	5.0	2555	0.167	IIA	
HSAV1A	Diazapam	6.8	2075	0.118	IIIA	
HDGX1A	Digitoxin	5.0	3751	0.137	IIIA,IA^IIA	
HCHLO1A	Chlofibrate	6.0	2175	0.138	IIIA	
HMRIB1A	Ibuprofen	6.0	2402	0.215	IIIA	
HAZT1A	AZT	4.0	7548	0.124	IIIA	

It should be noted that these data represent preliminary research into ligand binding of HSA. The binding results can also be affected by the composition of the solvent, the pH, and protein packing within the crystal. Where D = minimum d-spacing for data collected, N = number of unique reflections measured, Rf = agreement between native and ligand for Fobs.s

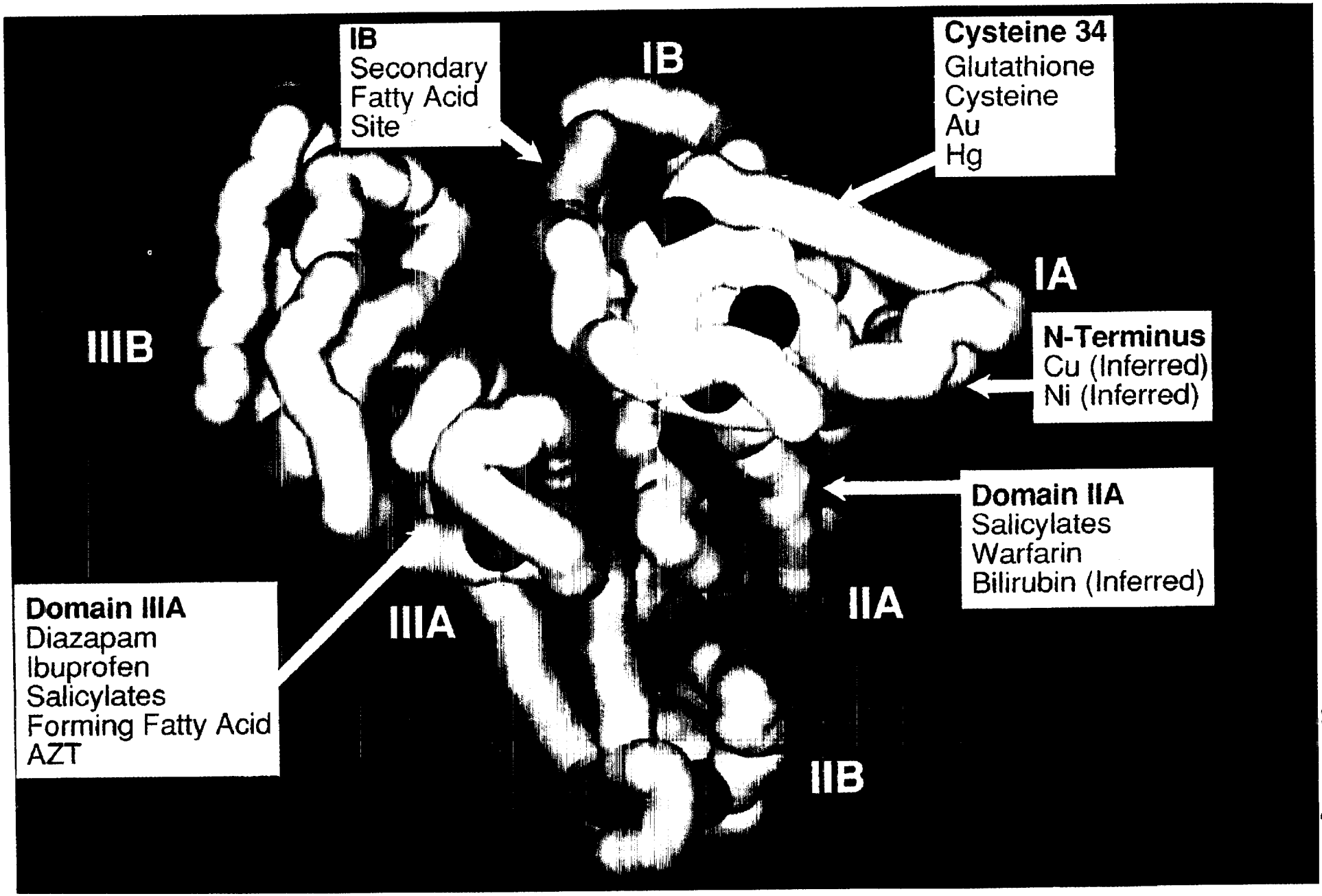

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FIGURE 1