Steroid-binding Specificity of Human Sex Hormone-binding Globulin Is Influenced by Occupancy of a Zinc-binding Site*

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One calcium-binding site (site I) and a second poorly defined metal-binding site (site II) have been observed previously within the amino-terminal laminin G-like domain (G domain) of human sex hormone-binding globulin (SHBG). By soaking crystals of this structure in 2.5 mM ZnCl₂, site I and a new metal-binding site (site III) were found to bind Zn²⁺. Site II is located close to the steroid-binding site, and Zn²⁺ is coordinated by the side chains of His⁸⁸ and His¹³⁸ and the carboxylate group of Asp⁶⁵. In this site, Zn²⁺ prevents Asp⁶⁵ from interacting with the steroid 17β-hydroxy group and alters the conformations of His⁸³ and His¹³⁸, as well as a disordered region over the steroid-binding site. Site III is formed by the side chains of His¹⁰¹ and the carboxylate group of Asp¹¹⁷, and the distance between them (2.7 Å) is increased to 3.7 Å in the presence of Zn²⁺. The affinity of SHBG for estradiol is reduced in the presence of 0.1–1 mM Zn²⁺, whereas its affinity for androgens is unchanged, and chemically-related metal ions (Cd²⁺ and Hg²⁺) have similar but less pronounced effects. This is not observed when Zn²⁺ coordination at site II is modified by substituting Gln for His¹³⁸. An alteration in the steroid-binding specificity of human SHBG by Zn²⁺ occupancy of site II may be relevant in male reproductive tissues where zinc concentrations are very high.

Plasma sex hormone-binding globulin (SHBG)¹ is a homodimeric glycoprotein produced by hepatocytes (1). It transports sex steroids in the blood and regulates their access to target cells (1, 2). The transcription unit responsible for plasma SHBG production by the liver is also expressed in rat Sertoli cells (3), and the protein product is known as the androgen-binding protein (ABP). Testicular ABP has exactly the same primary structure and steroid-binding properties as plasma SHBG and is thought to control androgen-dependent sperm maturation in the male reproductive tract (1, 3). Human SHBG and rat ABP comprise two laminin G-like domains (4) and share sequence similarity with the carboxyl-terminal regions of numerous extracellular proteins with diverse functions, including the blood coagulation co-factor, protein S; the growth factor, Gas 6; and several matrix-associated proteins (4). There is reason to suspect that the structural relationship between these proteins is functionally significant because their SHBG-like domains comprise binding sites for several members of an orphan tyrosine-kinase receptor superfamily (5). In addition, several groups have advanced the hypothesis that SHBG interacts with a plasma membrane receptor that contributes to steroid signaling pathways (6), but the biological impact of these interactions remains obscure.

Studies of human and rabbit SHBG with the luminescent lanthanide, terbium, have indicated that each dimer of SHBG contains four metal-binding sites (7). At least one of these sites was assumed to bind calcium because calcium ions stabilize the steroid-binding activity of the purified protein (8) and enhance SHBG dimer formation (9). A calcium-binding site was recently located within the amino-terminal laminin G-like domain (G domain) of human SHBG, which was complexed with steroid ligand, but it is at least 20 Å from the closest steroid atom and is not close to the proposed dimer interface (10). In addition, a strong difference density peak was observed at the end of strand β₆ in this crystal structure but was unsatisfactorily modeled as another calcium-binding site because of multiple conformations of the coordinating side chains (10).

The location of this second metal-binding site was intriguing because it lies in the vicinity of a disordered region in the crystal structure of the amino-terminal G domain of human SHBG, which loops over the steroid-binding site (10). Moreover, amino acids within this particular region of human SHBG can be affinity labeled with reactive groups attached to steroid ligands (11), and their substitution with residues in the corresponding position of rat ABP results in altered steroid-binding specificity (9). The possibility that the second metal-binding site might have been partially occupied by zinc in our original crystal structure (10) was considered because histidine residues are present at the end of strand β₆, as well as in the disordered loop over the steroid-binding site. Furthermore, the histidine at the end of strand β₆ is next to Asn³², which forms a hydrogen bond with the hydroxyl-group at C17 in the steroid ring D (10). This suggested to us that coordination of a metal ion, such as Zn²⁺, in this position might influence the steroid-binding activity of SHBG. There are large amounts of zinc in the male reproductive tract, especially in locations where SHBG may play a role in regulating the activities of sex-steroids, such as the prostate (12, 13). Therefore, we set out to determine if a metal-binding site exists in this region of human

Received for publication, May 24, 2000, and in revised form, June 15, 2000
Published, JBC Papers in Press, June 19, 2000, DOI 10.1074/jbc.M004484200

1 The abbreviations used are: SHBG, sex hormone-binding globulin; ABP, androgen-binding protein; DHT, 5α-dihydrotestosterone.

* This work was supported by grants from the Medical Research Council of Canada and the Deutsche Forschungsgemeinschaft. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The atomic coordinates and structure factors (code 1FSF) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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SHBG and if its occupancy by zinc influences the steroid-binding specificity of the protein.

**EXPERIMENTAL PROCEDURES**

**Crystallographic Analysis of Zinc-soaked SHBG Crystals**—Crystals of the amino-terminal G domain of SHBG were obtained as described (14). Single crystals were harvested from the crystallization droplets and soaked for three days in a “soaking solution” (5% polyethylene glycol 400 and 20% isopropanol in 100 mM HEPES buffer, pH 7.5, containing 1 mM CaCl₂, 1.5 mM 5α-dihydrotestosterone (DHT), and 2.5 mM ZnCl₂). Before flash cooling to prevent ice formation, the crystals were transferred for 5 min into the soaking solution to which 20% (v/v) glycerol had been added. A data set to 1.7 Å resolution was collected on beamline ID14 at the ESRF synchrotron in Grenoble at a wavelength of 0.93 Å using a MarCCD detector. A total of 120 exposures recorded in three different passes, with one and two degree oscillation steps each, and aiming at different resolution shells, yielded a 96.3% complete data set with a merging R-factor of 6.5% in program XDS (15). When keeping the Friedel pairs unmerged, the data set is still 95.3% complete (Table I).

The zinc-soaked crystals of SHBG are of space group R32 with cell axes a = 104.02 Å and c = 84.71 Å and are isomorphous to the crystals of the previously reported (10) SHBG model (Protein Data Bank accession number 1d2s). Structure refinement was straightforward, starting with a model consisting of only the polypeptide chain and the bound DHT, and alternating between rounds of visual inspection with program O (16) and refinement with program REFMAC (17). Initial Sigma-weighted difference density maps (18) clearly revealed the location of the metal ions, and the identity of the bound cations became apparent in a density map calculated with the anomalous differences and shifted model phases. The difference in the anomalous scattering between Zn²⁺ and Ca²⁺ (F₁-F₂, 0.93 Å) gave rise to 17.6 and 11.4 σ peaks at the two Zn²⁺ locations (sites I and II; see below) but only to a 5.0 σ peak at the Ca²⁺-binding site. In final refinement rounds, an explicit solvent model was included with program XPLOR (19) was introduced into REFMAC. The final model consists of residues 13–131 and 136–188 of SHBG, one molecule of DHT, and one Ca²⁺ and two Zn²⁺ ions (Table I). The model also includes 130 solvent molecules and one isopropanol molecule on top of a crystallographic 2-fold axis. The coordinates have been deposited with the Protein Data Bank (accession code 1FS5).

**Purification of Human SHBG**—Human SHBG was isolated from sheep-γ-transgenic mouse serum (20) as follows: the serum was diluted with an equal volume of 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, and (NH₄)₂SO₄ was added to 10% saturation at 0 °C. After centrifugation for 15 min at 5000 × g, the pellet and floating lipid were discarded. To the supernatant, (NH₄)₂SO₄ was added to 50% saturation at 0 °C. The mixture was centrifuged for 30 min at 10,000 × g, the pellet was redissolved in a minimum volume of water, and the solution was dialyzed overnight at 4 °C against 20 mM Tris-HCl, pH 8.0. The dialyzed solution was centrifuged to remove particulate material, and was loaded onto an HPLC protein liquid chromatography column packed with 8 ml of anion-exchange resin SOURCE 15Q (Amersham Pharmacia Biotech) and equilibrated with 20 mM Tris-HCl, pH 8.0, containing 1 mM testosteron. Elution was performed using a linear 0–0.25 M NaCl gradient. Fractions containing SHBG were identified using a steroid-binding capacity assay (21) and combined for dialysis prior to a second round of anion-exchange chromatography on a 1-ml SOURCE 15Q fast protein liquid chromatography column. Fractions (0.5 ml) containing SHBG were again combined, concentrated by ultrafiltration through an Ultrafree 15 centrifugal filter device (Millipore), and subjected to gel filtration on a fast protein liquid chromatography column packed with Superdex 75 and equilibrated with 10 mM Tris-HCl, pH 7.0, 1 M NaCl, 1 mM testosteron. The fractions containing SHBG were combined for final purification by preparative polyacrylamide gel electrophoresis using a Model 491 Prep Cell (Bio-Rad). The yield of pure SHBG was about 70%, and its physicochemical and steroid-binding properties were similar to those reported for SHBG isolated from human serum using steroid-ligand affinity chromatography (22).

**Production and Purification of Human SHBG Mutants**—The amino-terminal G domain (residues 1–205) of human SHBG was produced in Escherichia coli and purified as described earlier (14, 23). Wild-type human SHBG and a H136Q SHBG variant were expressed in Chinese hamster ovary cells (9), and serum-free medium from the cells was concentrated by ultrafiltration through PM-30 membranes (Amicon) with a simultaneous change in buffer to 50 mM Tris-HCl, pH 7.0, 100 mM NaCl, 0.1% NaNO₃.

Steady-state activity measurements—A conventional steroid-binding assay using [1H]DHT as the labeled ligand (21) was modified to assess the effect of calcium and additional interactions in the absence of Zn²⁺ or other divalent cations. To avoid formation of insoluble phosphates, 50 mM Tris-HCl, 100 mM NaCl, 1 mM CaCl₂ containing 0.1 mg/ml gelatin and 0.1% NaN₃ was used as the assay buffer. Equilibrium-binding parameters of SHBG and its mutants were determined by Scatchard analysis (24), and their binding affinities for testosterone or estradiol were assessed relative to DHT, as described previously (21). The steroid-binding properties of SHBG and its mutants were studied after incubation (10 min at room temperature) with various concentrations of different metal (II) chlorides. These steroid-binding assays were performed in the presence of the same salt at a three times lower concentration.

**RESULTS**

**Location of Two Zinc-Binding Sites within the Amino-terminal G Domain of Human SHBG**—Three different heavy atom-binding sites can be identified in the 1.7-Å structure of zinc-soaked crystals of the amino-terminal G domain of SHBG (Fig. 1). Site I is occupied by a calcium ion and is located on the rim of the β-sheet sandwich at the side opposite to the steroid-binding pocket. The Ca²⁺ is coordinated by seven oxygen atoms forming a pentagonal bipyramid. Two oxygen atoms are provided by the carboxylate group of Asp65 and His83 and other divalent cations. To avoid formation of insoluble phosphates, 50 mM Tris-HCl, 100 mM NaCl, 1 mM CaCl₂ containing 0.1 mg/ml gelatin and 0.1% NaNO₃ was used as the assay buffer. Equilibrium-binding parameters of SHBG and its mutants were determined by Scatchard analysis (24), and their binding affinities for testosterone or estradiol were assessed relative to DHT, as described previously (21). The steroid-binding properties of SHBG and its mutants were studied after incubation (10 min at room temperature) with various concentrations of different metal (II) chlorides. These steroid-binding assays were performed in the presence of the same salt at a three times lower concentration.

**TABLE I**

| Data Collection | Space group | R32 |
|-----------------|-------------|-----|
| Cell parameters a, c (Å) | 104.02, 84.71 |
| Resolution (Å) | 20–1.7 (1.8–1.7) |
| Number of reflections/unique reflections | 101,085/18,785 |
| Average I/σ(I) | 13.9 (2.8) |
| Completeness (%) | 96.3% (92.3) |
| Completeness of the anomalous data (%) | 95.3% (90.7) |
| Rmerge (%) | 6.5% (28.7) |
| **Model** | |
| Total number of residues | 172 |
| Content of the asymmetrical unit | |
| SHBG (residues 1–205) | 1 |
| DHT | 1 |
| Ca²⁺ | 1 |
| Zn²⁺ | 2 |
| H₂O | 130 |
| Isopropanol | 1 |
| Number of side chains with alternative conformations | 5 |
| Total non-H atoms | 1524 |
| Average B-factor (Å²) | 38.7 |
| **Diffraction agreement and stereochemistry** | |
| Resolution (Å) | 20–1.7 |
| R-value (%) | 19.7% (28.5) |
| Free R-value (%) | 25.9% (35.3) |
| r.m.s.d. in bonds (Å) | 0.013 |
| r.m.s.d. in angles (Å) | 0.035 |
| r.m.s.d. in temperature factors of bonded atoms | |
| Main chain (Å²) | 3.8 |
| Side chain (Å²) | 4.8 |

*Values for the highest resolution shell are given in parentheses. a, r.m.s.d., root mean square deviation from ideal values.

**Addendum**

The crystallographic coordinates and structure factors have been deposited in the Protein Data Bank (accession code 1FS5).
in the chain trace after residue 131. Residues 130 and 131 were not part of the previously published SHBG structure (10). In the zinc-soaked crystals, the phylogenetically conserved Leu\textsubscript{131} (1) is associated with high thermal displacement factors; it packs in between the side chains of Met\textsubscript{107} and Met\textsubscript{139} and points toward the hydrophobic portion of the steroid.

In the original crystal structure of the amino-terminal G domain of human SHBG (10), we observed a strong density at site II. Local disorder, and the existence of alternative conformations for the side chains of Asp\textsubscript{65} and His\textsubscript{83}, hinted that this site was only partially occupied in the crystals, and this prohibited the unambiguous identification of the bound metal ion (Fig. 2B). In the zinc-soaked crystals, no disorder is apparent at this position. The height of the crystallographic anomalous signal, as well as the nature of the coordinating ligands, clearly identifies this site as being occupied by zinc. As expected, the refined thermal displacement factor of the site II Zn\textsuperscript{2+} ion (40.2 Å\textsuperscript{2}) is comparable to those of the coordinating atoms (43.4 Å\textsuperscript{2} on average).

Metal-binding site III is formed by the side chain of His\textsubscript{101} from the intersheet crossing segment β\textsubscript{7} to β\textsubscript{8} and the carboxylate group of Asp\textsubscript{117} from the loop segment connecting strand β\textsubscript{9} to β\textsubscript{10} (Fig. 1). In the previously published SHBG structure, a 2.7 Å salt bridge is formed between these residues. Upon zinc binding, and as visible in the anomalous density map, zinc intercalates between the two side chains and increases the distance between the functional groups to 3.7 Å. Two additional solvent molecules participate in the distorted tetrahedral coor-
The ability of estradiol to compete with [3H]DHT for the SHBG steroid-binding site was assessed in the absence (–) or presence of 1 mM metal (II) chlorides, and expressed as B/B0, i.e., as a ratio of the amounts of [3H]DHT bound to SHBG in the presence (B) or absence (B0) of 1 μM estradiol. The results are the means of data obtained in two experiments; only Zn2+, Cd2+, and Hg2+ consistently inhibited the ability of estradiol to compete with [3H]DHT.

| Ion   | B/B0 (%) |
|-------|----------|
| Mg2+ | 18       |
| Ca2+ | 19       |
| Ni2+ | 20       |
| Cu2+ | 21       |
| Zn2+ | 55       |
| Cd2+ | 41       |
| Hg2+ | 36       |

Effect of Metal Ions on Human SHBG Steroid-binding Specificity—To investigate the effect of metal ions on the steroid-binding properties of SHBG, the purified protein was preincubated for 10 min at room temperature with various metal chlorides (as listed in Table II) at a 1 mM concentration prior to performing a [3H]DHT-binding assay. None of the cations influenced DHT binding to SHBG, but zinc and two chemically related metals, cadmium and mercury, inhibited the ability of estradiol to compete with the labeled ligand for the SHBG steroid-binding site (Table II). An independent experiment, in which [3H]estradiol was used as a tracer, confirmed that 1 mM zinc greatly reduces the affinity of SHBG for this estrogen (data not shown). A more detailed analysis (Fig. 3) revealed that the inhibitory effect of zinc on estradiol binding to SHBG could be detected at 10 μM ZnCl₂ and increases progressively with increasing ZnCl₂ concentrations. Similar effects of cadmium and mercury appeared at higher concentrations (100 μM to 1 mM) and were less pronounced (Fig. 3).

Analysis of dose-response curves for DHT, testosterone, and estradiol competition of [3H]DHT binding to purified SHBG (Fig. 4) further confirmed that the SHBG affinity for androgens was not influenced by zinc, whereas that for estradiol decreased ~6-fold. Similar results were obtained for SHBG in diluted human pregnancy serum, and in this case 1 mM ZnCl₂ caused a 3.5-fold reduction in the SHBG affinity for estradiol.

As described above, our crystallography data revealed two zinc-binding sites within the amino-terminal G domain of human SHBG. Although this domain seems to contain all the amino acids involved in steroid binding (10), the inhibitory effect of zinc on estradiol binding to SHBG might be mediated by additional metal-coordinating sites in the C-terminal G domain of SHBG via long distance conformational rearrangements. However, we observed that the inhibitory effect of zinc on estrogen binding to the amino-terminal G domain of human SHBG is even more pronounced and can be detected at submicromolar concentrations (Fig. 5). Moreover, very low concentrations of zinc (1–10 nM) produced a slight beneficial effect on both androgen and estrogen binding to this isolated domain (data not shown), presumably through stabilization of its structure. On the other hand, high concentrations of zinc (0.1–1 mM) may destabilize this truncated protein as evidenced by a slight decrease in DHT binding (Fig. 5). Nevertheless, competition data (Table III) show unequivocally that increasing ZnCl₂ concentrations from 10 nM to 500 μM alters the steroid-binding specificity of the amino-terminal G domain of human SHBG, i.e., its relative binding affinity for estradiol was approximately five times lower at the highest ZnCl₂ concentration, whereas that for testosterone was increased 1.5-fold.

Identification of the Zinc-binding Site That Influences the Steroid-binding Specificity of SHBG—Expression of wild type human SHBG and a H136Q human SHBG variant (9) allowed us to ascertain which of the two zinc-binding sites identified in...
the crystal structure has the ability to alter the steroid-binding specificity of human SHBG. The steroid-binding specificity of human SHBG produced by Chinese hamster ovary cells was affected by zinc in almost exactly the same way as SHBG in human serum, i.e. there was an ~5-fold reduction in affinity for estradiol in the presence of 1 mM ZnCl₂, whereas the ability of estradiol to displace [³H]DHT from the H136Q SHBG variant (9) was not influenced by zinc at all. Furthermore, sheep SHBG also contains a Gln instead of His at position 136 (1), and analysis of purified sheep SHBG indicated that zinc has no effect on its steroid-binding specificity (data not shown).

**DISCUSSION**

We have identified three binding sites for metal ions in the amino-terminal G domain of human SHBG. Site I binds calcium, whereas sites II and III bind zinc. The bound metal ion in each site can be identified unambiguously based on the geometry and nature of the coordinating atoms, as well as the height of the anomalous signal in the electron density map. Based on the present data, and a previous estimate that each SHBG subunit contains two potential calcium-binding sites (7), the carboxyl-terminal G domain of human SHBG probably also contains a calcium-binding site, which seems to be a recurring feature of laminin G-like domain structures (25).

It is known that Ca²⁺ stabilizes the steroid-binding properties of purified SHBG (8), and Ca²⁺ and Zn²⁺ promote SHBG dimer formation (9). Of all three metal sites, site III is closest to the proposed dimer interface formed by residues from strands β7 and β10 (10). This site might therefore be responsible for the stabilization of dimer formation, but the changes observed in the presence or absence of zinc in the vicinity of site III are minor and are limited to a widening of the salt bridge between His₁³⁶ and Asp₁¹⁷. Therefore, we conclude that the zinc effect on dimer formation is the result of an overall stabilization of the monomer, rather than allosteric rearrangements or an immediate involvement of zinc at the dimer interface.

In a number of zinc-containing proteins, such as carboxypeptidase A, carboanhydrase, and alkaline phosphatase, Zn²⁺ can be substituted with Hg²⁺, Cd²⁺, Cu²⁺, Ni²⁺, and Co²⁺, but in most cases this abolishes enzyme activity (26). In the case of SHBG, alterations in its affinity for specific steroids are only observed in the presence of metal ions when metal-binding site II was partially occupied (see Fig. 2B). Our studies of an SHBG deletion mutant confirm that the zinc effect on SHBG steroid-binding specificity is mediated by metal-binding sites in its amino-terminal G domain. To identify which of the two zinc-binding sites in this domain is responsible for alterations in steroid-binding specificity, we used an SHBG variant in which the His at position 136 is substituted for a Glu (9). This substitution was chosen because it has a minor effect on the steroid-binding specificity of SHBG (9), and because the crystallography data indicated that His₁³⁶ plays a key role in coordinating zinc in site II. Our data clearly indicate that this substitution eliminates the zinc-induced reduction in SHBG affinity for estradiol and lead us to conclude that zinc exerts its effect on the steroid-binding specificity of SHBG via metal-binding site II. We propose that this can be explained by the following mechanism. In the absence of zinc, Asp₆⁵ participates in sterosteroid binding by making a hydrogen bond with the oxygen atom of the hydroxy group at C17 of the steroid, and the side chain of Asp₆⁵ is further held in place by a hydrogen bond to the side chain of Thr₁⁶⁰ (Fig. 6). Upon zinc binding, this network of hydrogen bonds becomes disrupted because the side chain of Asp₆⁵ now turns outwards and participates together with His₁³⁶ and His₁³⁶ in zinc binding. A slight displacement of the side chain of Thr₁⁶⁰ also takes place, whereas the hydrogen bond between Asn₁³⁵ and the hydroxy-group at C17 of DHT remains remarkably unaffected. This is because an almost identical length is observed for this hydrogen bond, namely 2.93 and 2.89 Å, in both structures (Fig. 6). However, these changes in hydrogen bonding of the steroid only affect the affinity of SHBG for estradiol. This indicates that the relative importance of the hydrogen bonds between Asn₁³⁵ and Asp₆⁵ and the hydroxy group at C17 of the steroid molecule differs markedly between C₁₈ and C₁₉ steroid hormones and might be because of small differences in steroid orientation in the binding pocket caused by the absence of the C19 methyl group and/or the aromatic nature of ring A in estradiol. Although this is a reasonable assumption, it needs to be confirmed by further crystallographic analysis of the SHBG steroid-binding site in association with estradiol.

The geometry of the zinc-binding site II is very similar to the

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**Table III**

| ZincCl₂ | DHT | Testosterone | Estradiol |
|--------|-----|--------------|-----------|
| µM    | IC₅₀ for unlabeled competitors | nM  |           |          |
| 0.01  | 19  | 110          | 270       |
| 50    | 19  | 76           | 380       |
| 500   | 17  | 73           | 760       |
zinc-binding site in carboxypeptidase A (26) except that glutamic acid is replaced by aspartic acid in the case of SHBG. This is intriguing because when the amino-terminal G domain of human SHBG is expressed as a glutathione S-transferase fusion protein and purified, it undergoes proteolysis and release from its fusion partner upon storage (23). It is not known whether this phenomenon has biological relevance, but the first 12 residues of the purified amino-terminal G domain of SHBG used for crystallography are also lost during storage, and are presumably removed by proteolysis.

Several different crystal structures of proteins containing G domains with a very similar fold have been published recently (10, 30, 31). It is interesting that protein-protein interaction sites mapped onto the surface of individual G domains coincide with the steroid-binding region of SHBG. In addition, a Ca2+ ion is bound in laminin G5 at a position that is virtually identical to site II of SHBG, and it has been proposed that this is responsible for the calcium dependence of laminin binding to dystroglycan (31). In light of what appears to be a conserved multifunctional interaction site in these G domains and the rather complex mechanism revealed here for the modulation of the steroid-binding specificity of SHBG by Zn2+, this region may also be involved in the interaction between SHBG and proteins on the surface of some cells (6).

The histidine at position 136, which plays an important role in coordinating zinc in the metal-binding site II, is not conserved in SHBG molecules from a variety of other mammalian species (1). In addition, it is located in a poorly conserved region that spans the disordered loop structure over the steroid-binding site in our human SHBG crystal structures. Sheep SHBG not only lacks a histidine in this region but has an N-glycosylation site that is utilized at a position corresponding to Ser133 in human SHBG (1). However, when compared with SHBG from many other subprimate species, sheep SHBG has a relatively high affinity for estradiol, which is not influenced by the presence of zinc. In contrast, rabbit SHBG and rodent ABPs containing a histidine residue within this region, and it remains to be seen whether their steroid-binding properties are also influenced by the presence of zinc. Species-specific effects of zinc on endocrine functions are not without precedent. For example, the binding of human growth hormone to the prolactin receptor is increased remarkably in the presence of zinc (32). As shown by mutational and structural studies, the His136, which participates in zinc binding to human growth hormone is conserved only in primates (33), and this explains why growth hormone is not lactogenic in nonprimate species (32, 33).

Even if the zinc-dependent alteration in the steroid-binding specificity of human SHBG is not conserved in other mammalian species, it may serve some specialized function in human tissues where zinc concentrations are high and where SHBG is sequestered from the blood circulation, such as the prostate stroma (12, 13). In this regard, benign prostatic hyperplasia in humans is associated with an excessive activity of sex steroids and/or imbalance in their relative activities in the stromal compartment of the prostate (34). It remains to be defined how sex steroids are linked to the genesis of this disease but the levels of estradiol are abnormally high in hyperplastic human prostate stroma (35), and this may in some way be related to a local alteration in the binding affinity of SHBG for estradiol versus androgens.

Acknowledgments—We thank Irina Grishkovskaya for crystals of SHBG, David Dales and Maria Catalano for assistance in the production of SHBG mutants, Heather Hodgett-Jury for the transgenic mouse blood, Ed Mitchell from the European Synchrotron Radiation Facility for help with data collection, Udo Heinemann from the Max-Delbrück Center for generous support, and Denise Power for secretarial help.

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J. Biol. Chem. 2000, 275:25920-25925.
doi: 10.1074/jbc.M004484200 originally published online June 19, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M004484200

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