Crystal Structure of Human Sex Hormone-binding Globulin in Complex with 2-Methoxyestradiol Reveals the Molecular Basis for High Affinity Interactions with C-2 Derivatives of Estradiol*

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In a crystal structure of the amino-terminal laminin G-like domain of human sex hormone-binding globulin (SHBG), the biologically active estrogen metabolite, 2-methoxyestradiol (2-MeOE2), binds in the same orientation as estradiol. The high affinity of SHBG for 2-MeOE2 relies primarily on hydrogen bonding between the hydroxyl at C-3 of 2-MeOE2 and Asp65 and an interaction between the methoxy group at C-2 and the amido group of Asn82. Accommodation of the 2-MeOE2 methoxy group causes an outward displacement of residues Ser128–Pro130, which appears to disorder and displace the loop region (Leu131–His139) that covers the steroid-binding site. This could influence the binding kinetics of 2-MeOE2 and/or facilitate ligand-dependent interactions between SHBG and other proteins. Occupancy of a zinc-binding site reduces the affinity of SHBG for 2-MeOE2 and estradiol in the same way. The higher affinity of SHBG for estradiol derivatives with a halogen atom at C-2 is due to either enhanced hydrogen bonding between the hydroxyl at C-3 and Asp65 (2-fluoroestradiol) or accommodation of the functional group at C-2 (2-bromoestradiol), rather than an interaction with Asn82. By contrast, the low affinity of SHBG for 2-hydroxyestradiol can be attributed to intra-molecular hydrogen bonding between the hydroxyls in the aromatic steroid ring A, which generates a steric clash with the amido group of Asn82. Understanding how C-2 derivatives of estradiol interact with SHBG could facilitate the design of biologically active synthetic estrogens.

Sex hormone-binding globulin (SHBG) is the major plasma transport protein for biologically active sex steroids, and changes in its plasma concentrations influence the plasma half-lives of their steroid ligands and their access to target tissues (1, 2). Human SHBG is a homodimeric glycoprotein, and each monomer contains a steroid-binding site within an amino-terminal laminin G-like (LG) domain (3, 4). Biologically active androgens and estradiol compete for occupancy of this binding site, but these two classes of sex steroids appear to enter the site quite differently and are bound in opposite orientations (5). Occupancy of the human SHBG steroid-binding site by estradiol is also accompanied by a specific positioning and/or conformation of amino acid residues on the surface of the protein that is not seen when the site is occupied by C19 steroids or the synthetic progestin, levonorgestrel (5). Such ligand-dependent alterations in the tertiary structure of SHBG are of interest in light of reports that it interacts with binding sites on the plasma membranes of different tissues in either a ligand-dependent (6) or ligand-specific (7) manner. The biological significance of these interactions remains poorly defined, but they may be implicated in rapid non-genomic actions of steroids (8).

Human SHBG binds several androgen and estrogen metabolites (9) and has a higher affinity for 2-methoxyestradiol (2-MeOE2) than for estradiol or testosterone (1). The presence of a halogen atom at C-2 of the estradiol molecule is also known to increase the affinity of ligand binding to SHBG (10), whereas a hydroxy group at the same position has the opposite effect (1). The binding of 2-MeOE2 is of particular interest because this estrogen metabolite is biologically active, and appears to exert its effects via non-genomic mechanisms. For instance, 2-MeOE2 interacts with the colchicine-binding site of tubulin and inhibits its polymerization (11). There is also increasing evidence that 2-MeOE2 acts on the vascular endothelium to inhibit blood vessel formation (12), and binding to plasma SHBG can therefore be expected to play a key role in regulating the amounts of biologically active 2-MeOE2 in the capillaries of tissues containing rapidly proliferating cells. It has also been reported that 2-MeOE2 binding to human SHBG in complex with its putative plasma membrane receptor fails to produce a signal transduction event similar to that observed when other steroids interact with plasma membrane-bound SHBG (8). The reason for this is obscure, but it may be related to specific ligand-dependent conformational changes in SHBG, as noted recently (5).

We have now obtained a crystal structure of the amino-terminal LG domain of human SHBG in complex with 2-MeOE2, and we have combined this information with site-directed mutagenesis experiments to define the molecular basis of high affinity interactions between SHBG and estradiol derivatives with substitutions at C-2 of the steroid ring A.
view of our previous observations that occupancy of a zinc-binding site at the entrance of the human SHBG steroid-binding pocket specifically influences its ability to interact with estradiol (5), we have also explored how this might affect the binding of 2-MeOE2.

**EXPERIMENTAL PROCEDURES**

**Crystal Production and Analysis**—The amino-terminal LG domain of human SHBG was expressed, purified, and crystallized, as described previously (13), with the exception that 2-MeOE2 was used instead of 5α-dihydrotestosterone (DHT) to saturate the steroid-binding site during all steps. Crystallization was accomplished using the hanging drop method. In brief, 1 μl of protein solution (13 mg/ml protein, 50 mM Na-HEPES, pH 7.5, 2.5 mM CaCl₂) containing 10 μl 2-MeOE2 (Steroids Inc.) was mixed with 1 μl of reservoir solution (20% isopropanol alcohol, 10% polyethylene glycol 400, 100 mM CaCl₂ in 100 mM Na-HEPES, pH 7.5). This crystallization mixture was equilibrated against 1 ml of reservoir solution at 20 °C, and crystals of SHBG in complex with 2-MeOE2 were obtained within 1 week. Before flash cooling, crystals were transferred into a soaking solution containing 30% isopropanol alcohol, 10% polyethylene glycol 400 in 100 mM Na-HEPES, pH 7.5, for 5 min to prevent ice formation. A complete data set of SHBG bound to 2-MeOE2 was collected at 1.75 Å resolution at beam line BW7 of the EMBL outstation at the DESY synchrotron in Hamburg, Germany. Data were reduced with the program package DENZO/SCALEPACK (14), and the crystal structure was solved by difference Fourier techniques using the protein atomic coordinates for SHBG in complex with DHT from the Protein Data Bank (accession code 1D2S). To obtain target geometry parameters for the ligand (2-MeOE2) during crystallographic refinement, an idealized model was built by merging the crystal structure of 2,3-dimethoxyestradiol retrieved from the Cambridge structural data base (15) with our previous estradiol model (5). From these coordinates, a topology and parameter file was generated using the Hic-up (x-ray.bmc.uu.se/hicup) server (16). The structure of the complex was subjected to several rounds of refinement by molecular dynamics and energy minimization using the program CNS (17), alternating with manual inspection in program O (18). The free R-factor reflections were identical to those used previously to monitor the refinement of SHBG steroid complexes (3–5). The final model and the experimental structure factor amplitudes have been deposited with the Protein Data Bank.

**Production and Analysis of Human SHBG Variants**—Apart from the S128A SHBG variant, all the human SHBG variants used to assess the contribution of specific amino acids to the binding affinities for various steroid ligands were produced as described previously (5). The S128A SHBG variant was produced by site-directed mutagenesis (5) by using the following oligonucleotide sequence with the altered bases within the codon for Ser128 underlined: 5'-GGTACGGGCCCAGCGACCTGTCT-3'. The amino-terminal LG domain of human SHBG purified from serum (5) or recombinant wild-type human SHBG previously (13), with the exception that 2-MeOE2 was used instead of 17β-estradiol in the crystallization mixture was equilibrated against 1 ml of reservoir solution at 20 °C, and crystals of SHBG in complex with 2-MeOE2 were obtained within 1 week. Before flash cooling, crystals were transferred into a soaking solution containing 30% isopropanol alcohol, 10% polyethylene glycol 400 in 100 mM Na-HEPES, pH 7.5, for 5 min to prevent ice formation. A complete data set of SHBG bound to 2-MeOE2 was collected at 1.75 Å resolution at beam line BW7 of the EMBL outstation at the DESY synchrotron in Hamburg, Germany. Data were reduced with the program package DENZO/SCALEPACK (14), and the crystal structure was solved by difference Fourier techniques using the protein atomic coordinates for SHBG in complex with DHT from the Protein Data Bank (accession code 1D2S). To obtain target geometry parameters for the ligand (2-MeOE2) during crystallographic refinement, an idealized model was built by merging the crystal structure of 2,3-dimethoxyestradiol retrieved from the Cambridge structural data base (15) with our previous estradiol model (5). From these coordinates, a topology and parameter file was generated using the Hic-up (x-ray.bmc.uu.se/hicup) server (16). The structure of the complex was subjected to several rounds of refinement by molecular dynamics and energy minimization using the program CNS (17), alternating with manual inspection in program O (18). The free R-factor reflections were identical to those used previously to monitor the refinement of SHBG steroid complexes (3–5). The final model and the experimental structure factor amplitudes have been deposited with the Protein Data Bank.

**RESULTS AND DISCUSSION**

**Crystal Structure of Human SHBG in Complex with 2-MeOE2**—The structure of the amino-terminal LG domain of human SHBG in a complex with 2-MeOE2 has been solved at 1.75 Å resolution and refined to convergence to a crystallographic R-factor of 21.0% (RFree = 23.8%, see Table I). As in the corresponding estradiol-bound SHBG crystal structure (5), 2-MeOE2 is oriented within the steroid-binding site so that its hydroxy group at C-3 is capable of interacting with Asp⁶⁸, whereas the hydroxy group at C-17 can interact with Ser⁶⁴ and the carbonyl oxygen of Valⁱ⁰⁵ (Fig. 1A). Thus, this binding mode appears to be typical of C18 steroids and contrasts with that of C19 steroids and the synthetic progesterin, levonorgestrel, which reside within the SHBG steroid-binding site in the opposite orientation (5).

Although the main chain atoms of the 2-MeOE2-bound SHBG crystal structure can be superimposed on the crystal structures of SHBG in complex with estradiol or DHT with average root mean square deviations as low as 0.59 and 0.68 Å, respectively, the binding of 2-MeOE2 results in distinct conformational differences within the amino-terminal LG domain of SHBG. For instance, the methyl group of 2-MeOE2 points into a hydrophobic pocket lined by residues Met³⁰⁷, Val¹⁺², Leu¹⁺⁴, and Val¹⁺⁷ (Fig. 1A), and its accommodation within this pocket (Fig. 1B) appears to affect conformation of the loop segment (Leu¹⁺¹–His¹⁺³) that covers the SHBG steroid-binding site (22). Accordingly, the Cα atoms of residues that precede this loop segment (Ser⁵⁺⁸, Gly¹⁺⁰, and Pro¹⁺⁹) are in close proximity to the methoxy group and are pushed away from the binding site by 1.0, 1.8, and 4.8 Å, respectively, when compared with the SHBG-estradiol complex (5). Furthermore, residues Leu¹⁺¹–Ser¹⁺³ are disordered in the SHBG complex with 2-MeOE2, and Lys¹⁺⁴ and Arg¹⁺⁵ are displaced when compared with the estradiol-bound SHBG complex (5) by 2.1 and 3.4 Å, respectively (Fig. 1B). In addition, the side chain of Trp⁵⁴ is oriented toward the steroid in the SHBG complex with 2-MeOE2 (Fig. 1A), which is similar to SHBG complexes with C19 steroids (3, 5) but quite different from that observed in the SHBG-estradiol complex (5). On the other hand, the region Trp⁷⁰–Lys⁷³ in the 2-MeOE2-bound SHBG structure closely follows the trace of the main chain of the estradiol-bound SHBG and diverges from the conformation observed in SHBG in complex with C19 steroids or levonorgestrel (5). This directly reflects the orientation of the steroid ligand in the binding site because it results from a van der Waals interaction between the C18 methyl group of estradiol or 2-MeOE2 with the side chain of Leu⁷¹, and this interaction does not exist in the complexes with C19 steroids. From this, we conclude that some structural alignments in the 2-MeOE2-bound SHBG are shared with other SHBG-steroid complexes; nevertheless, the binding of 2-MeOE2 results in several unique structural features within the amino-terminal LG domain of human SHBG.

The residue Asp⁶⁸ is a key player in the coordination of estradiol within the SHBG steroid-binding site (5) and most likely participates in hydrogen bonding with the hydroxyl at C-3 of 2-MeOE2 (Fig. 1A). This is supported by the fact that the distance (2.48 Å) between this hydroxy group and the carboxy-

### Table I

| Data collection | Resolution (Å) | 20–1.75 (1.79–1.75) |
|----------------|---------------|----------------------|
| Space group    | R32           |                      |
| Cell parameter a, c (Å) | 103.92, 84.46 |
| Number of reflections | 123,818 |
| Unique reflections | 17,701 |
| Completeness (%) (overall/outer shell) | 99.4/96.9 |
| Rsym (%) (overall/outer shell) | 3.1/28.6 |
| Refinement statistics |                      |
| Resolution (Å) | 20–1.75 |
| No. residues | 174 |
| No. water molecules | 97 |
| No. non-hydrogen atoms | 1501 |
| Average B-factor (Å²) | 38.5 |
| R value (%) | 21.0 |
| Rfree value (%) | 23.8 |
| Root mean square deviations | 0.010 |
| (bond lengths in Å) | 1.52 |
| Root mean square deviations | |
ylate group of Asp<sup>65</sup> in the 2-MeOE2-bound SHBG structure is increased by only 0.1 Å, when compared with the SHBG-estra
diol complex (5). Although Asn<sup>82</sup> is within hydrogen bonding
distance from the hydroxyl at C-3 of either estradiol or 2-MeOE2 in their respective SHBG complexes, we have shown
previously (5) by site-directed mutagenesis that its contribu-

### Table II

Comparison of the dissociation constants (K<sub>d</sub>) for DHT and the relative binding affinities (RBA) for estradiol and 2-MeOE2 of wild-type human SHBG and human SHBG variants produced by site-directed mutagenesis showing the fold increases (↑) or decreases (↓) when compared with wild-type SHBG values

| SHBG   | K<sub>d</sub> for DHT at 4 °C | Estradiol | 2-MeOE2 |
|--------|-----------------------------|-----------|---------|
| Wild type | 0.9 ± 0.2                   | 16.7 ± 2.8 | 6.9 ± 2.7 |
| S42A   | 16.8 ± 3.6<sup>↓</sup> (↑ 19x) | 6.8 ± 1.5<sup>↑</sup> (↑ 3x) | 0.8 ± 0.2<sup>↓</sup> (↑ 9x) |
| G58A   | 26.3 ± 6.2<sup>↑</sup> (↑ 29x) | 8.4 ± 2.6<sup>↑</sup> (↑ 2x) | 3.5 ± 0.6<sup>↑</sup> (↑ 2x) |
| D65A   | 0.8 ± 0.2                   | 18.8 ± 4.7<sup>↑</sup> (↑ 12x) | 57.3 ± 11.9<sup>↑</sup> (↑ 9x) |
| N92A   | 0.8 ± 0.2                   | 16.3 ± 2.6  | 23.2 ± 2.0<sup>↑</sup> (↑ 4x) |
| W84A   | 2.1 ± 0.7<sup>↑</sup> (↑ 2x) | 4.3 ± 0.5<sup>↑</sup> (↑ 4x) | 3.2 ± 0.4<sup>↑</sup> (↑ 2x) |
| M107A  | 1.0 ± 0.3                   | 15.6 ± 3.0  | 2.8 ± 0.9<sup>↑</sup> (↑ 3x) |
| S128A  | 1.4 ± 0.2                   | 15.2 ± 1.9  | 5.9 ± 1.5  |
| P130G  | 1.3 ± 0.1                   | 8.7 ± 1.2<sup>↑</sup> (↑ 2x) | 3.8 ± 1.3<sup>↑</sup> (↑ 2x) |
| L131G  | 14.6 ± 2.3<sup>↑</sup> (↑ 16x) | 7.8 ± 1.8<sup>↑</sup> (↑ 2x) | 0.6 ± 0.1<sup>↑</sup> (↑ 12x) |
| K134A  | 1.6 ± 0.5                   | 20.7 ± 6.2  | 6.7 ± 0.3  |
| H136Q  | 1.2 ± 0.1                   | 26.9 ± 3.2<sup>↑</sup> (↑ 2x) | 5.6 ± 1.4  |
| P137G  | 1.0 ± 0.2                   | 11.1 ± 1.5<sup>↑</sup> (↑ 2x) | 2.2 ± 0.1<sup>↑</sup> (↑ 3x) |

<sup>a</sup> Determined as a ratio of the concentration of steroid competitor resulting in a 50% reduction in specific binding of [3H]DHT to the concentration of DHT required to produce the same effect. Except for S128A, data for the K<sub>d</sub> of DHT and the RBAs of estradiol are from Ref. 5.

<sup>b</sup> Significantly different (p < 0.05) from the corresponding parameter of wild-type SHBG.

FIG. 1. Stereo representation of the human SHBG in complex with 2-MeOE2. A, details of the interaction between SHBG and 2-MeOE2 (in orange). The dashed lines indicate possible hydrogen bonds between functional groups at C-2, C-3, and C-17 of 2-MeOE2 and the corresponding amino acid side chains. The numbering of carbon atoms in the steroid molecule follows the IUPAC-IUB guidelines; the methoxy group at C-2 can hydrogen-bond to Asn<sup>82</sup>; the hydroxyl at C-3 can hydrogen-bond to Asp<sup>65</sup> and Asn<sup>82</sup>, and the hydroxyl at C-17 can hydrogen-bond to Ser<sup>132</sup> and the carbonyl oxygen of Val<sup>105</sup>. B, the conformation of the loop segment that covers the steroid-binding site in the 2-MeOE2 complex (in orange) differs from that observed in SHBG in complex with estradiol (in red, accession code 1LHU) and DHT (in blue, accession code 1KDK). No electron density is observed for residues Leu<sup>121</sup>, Thr<sup>122</sup>, and Ser<sup>132</sup> in the 2-MeOE2 complex. The figures were generated using Molscript (30) and Raster3d (31).
Fig. 2. Effect of zinc on the binding of estradiol and 2-MeOE2 to wild-type human SHBG and SHBG variants. Dose-response curves for the competition of $[^3H]$DHT binding to wild-type human SHBG (A) and the H136Q (B) and D65A (C) SHBG variants with increasing concentrations of DHT (circles), estradiol (squares), and 2-methoxyestradiol (triangles) in the absence of zinc (solid symbols and lines) and in the presence of 0.5 mM ZnCl$_2$ (open symbols and dashed lines). $B$ and $B_0$ are the amounts of the $[^3H]$DHT tracer specifically bound to human SHBG in the presence and absence of a steroid competitor, respectively.
tion to the binding affinity of SHBG for estradiol is negligible. This is probably also the case for the SHBG complex with 2-MeOE2 because the distance between Asn82 and the hydroxyl at C-3 of the steroid is increased by 0.2 Å, as compared with the estradiol-bound SHBG structure (5). On the other hand, the oxygen atom of the 2-MeOE2 methoxy group is only 3.0 Å from the Asn82 amido group (Fig. 1A), and the possibility of an interaction between these two functional groups exists.

Contributions of Specific Amino Acid Residues to the High Affinity of Human SHBG for 2-MeOE2—Previous studies (3, 5) have shown that functional groups at positions C-3 and C-17 of steroid ligands interact with Ser42 and Asp65 depending on their orientation within the SHBG steroid-binding site. The contribution of these residues to the high affinity binding of 2-MeOE2 was evaluated by comparing its ability to bind S42A and D65A SHBG variants with those of estradiol and DHT (5). As shown in Table II, the binding of 2-MeOE2 to S42A is less affected when compared with estradiol. In this context, it is important to appreciate that although estradiol and 2-MeOE2 compete more effectively with DHT for binding to the S42A SHBG variant, its affinity for DHT is greatly reduced (Table II). Thus, the net result of this amino acid substitution on estradiol and 2-MeOE2 binding to SHBG is also negative. By contrast, substitution of Asp65 with alanine has no effect on DHT-binding affinity but causes a substantial decrease in the

| SHBG          | RBAa         | 2-Fluoroestradiol | 2-Bromoestradiol |
|---------------|--------------|-------------------|------------------|
| Wild type     | 1.8 ± 0.3    | 0.7 ± 0.1         |
| D65A          | 57.8 ± 6.8²  | 5.1 ± 0.3³        |
| N82A          | 3.3 ± 0.4³   | 1.4 ± 0.2³        |

* Determined as a ratio of the concentration of steroid competitor resulting in a 50% reduction in specific binding of [3H]DHT to the concentration of DHT required to produce the same effect.

² Significantly different (p < 0.05) from the corresponding parameter of wild-type SHBG.

binding affinity of both estradiol and 2-MeOE2 (Table II). This is in line with our previous conclusion that this residue plays a much more important role in the binding of C18 steroids than C19 steroids (5). Crystal structures of human SHBG in complex with other steroid ligands have indicated that Asn82 might also contribute to their coordination within the steroid-binding site, but substitution of this residue does not influence the binding of C19 steroids, levonorgestrel or estradiol (3, 5). This is shown again in Table II. However, the binding affinity of 2-MeOE2 to the N82A SHBG variant is significantly reduced. This is in accordance with the crystal structure data indicating that the methoxy group of 2-MeOE2 can interact with the amido group of Asn82 and thereby contributes to the high affinity of human SHBG for 2-MeOE2.

The crystal structure of 2-MeOE2-bound SHBG indicates that the methoxy group protrudes into a pocket that includes Met107 (Fig. 1A), and when this residue was substituted with alanine the resulting SHBG variant displayed a specific increase in affinity for 2-MeOE2 (Table II). In view of the displacement of residues from the loop segment Leu132 to His136 (see above), these data imply that the accommodation of the methoxy group generates steric clashes in this region of SHBG.
which are attenuated by the replacement of Met^{107} with a smaller amino acid, such as alanine. Deeper within the SHBG steroid-binding site, Gly^{58} is located in such close proximity to steroid ligands that any substitution at this position will likely cause a steric clash and a marked reduction in steroid binding (3). As in the case of estradiol (5), the binding of 2-MeOE2 to the G58A SHBG variant is affected to a lesser extent than that of DHT (Table II), but overall this amino acid substitution still results in an ~15-fold reduction in binding affinity for these C18 steroids. The difference in the reduction of the relative affinities of the G58A SHBG variant for C18 and C19 steroids, as compared with wild-type SHBG, probably reflects the difference in their orientation within the SHBG steroid-binding site. In the SHBG complex with 2-MeOE2, the loop segment (Leu^{131}–His^{136}) that covers the steroid-binding site appears to be displaced when compared with the estradiol-bound SHBG structure reported previously (Fig. 1B). This loop segment is flanked by prolines at positions 130 and 137 that appear to control its flexibility (22). When these proline residues are substituted with glycine to augment the flexibility of this loop segment, this increases the affinity for 2-MeOE2 (Table II), as shown previously for estradiol (5). This confirms that the flexibility of the loop segment influences the binding of C18 steroids, and supports the hypothesis that this region of the polypeptide chain influences the ability of different classes of steroids to either enter or exit the binding site.

Although the methoxy group of 2-MeOE2 appears to push away residues adjacent to the loop segment that covers the steroid-binding site, such as Ser^{128}, substitution of this residue with alanine has no effect on the affinity of SHBG for 2-MeOE2 or other steroids (Table II). Within the loop region itself, Leu^{131} influences the binding of C19 steroids and estradiol (5). However, our present data indicate that Leu^{131} has little impact on the binding of 2-MeOE2 (Table II), and this is in agreement with the observation that Leu^{131}, Thr^{132}, and Ser^{133} are disordered in the crystal structure (Fig. 1B). Moreover, substitution of other residues within the loop region (e.g. Lys^{134} or His^{136}) had little or no effect on the binding of 2-MeOE2 (Table II). This was expected because in the 2-MeOE2-bound SHBG crystal structure the chain segment Lys^{134}–His^{136} is associated with high thermal displacement factors, which indicates that this region packs only loosely against the steroid-binding site. The steroid ligand-specific rearrangement of this loop segment may, however, have implications with respect to possible interactions between SHBG and other proteins, such as a specific receptor that has been reported to reside within the plasma membranes of some tissues (6, 7). If so, this would be in line with the concept that this region of the molecule represents a conserved interaction surface within a family of functionally highly diverse proteins that share the LG domain fold (23).

The orientation of the Trp^{84} side chain in the 2-MeOE2-bound SHBG structure (Fig. 1A) also differs from its outward orientation in estradiol-bound SHBG (5). The affinity of the W84A SHBG variant for 2-MeOE2 is essentially unchanged in relation to DHT, whereas its net affinity for estradiol increases 2-fold (Table II). This reflects the fact that the orientation of the side chain of Trp^{84} in the DHT and 2-MeOE2 complexes differs from that in the estradiol complex. The higher affinity of the W84A SHBG variant for estradiol can be attributed to an energetically disfavored orientation of Trp^{84} in wild-type SHBG caused by the inward movement of the residues (Ser^{128}, Gly^{129}, and Pro^{130}) preceding the loop segment Leu^{131}–His^{136} (5). In contrast, the methoxy group of 2-MeOE2 pushes these residues (Ser^{128}–Pro^{130}) outward upon occupancy of the SHBG steroid-binding site, and this circumvents the need for a reorientation of Trp^{84} (Fig. 1A). These observations further support the concept that the outward position of Trp^{84} represents a unique feature of the surface topology of estradiol-bound SHBG that could provide the basis for its recognition by other molecules (5).

Influence of Zinc on the Binding of 2-MeOE2 to SHBG—Both Asp^{65} and His^{136} play key roles in coordinating a zinc ion at the entrance of the steroid-binding site, and occupancy of this zinc-binding site causes a marked reorientation of the side chains of these residues (21). This in turn appears to alter the conformation of the loop segment that covers the steroid-binding site (22), and the combined effects cause a reduction in the binding affinity of human SHBG for estradiol, as compared with DHT or other C19 steroids (21). It is therefore of interest that the affinity of wild-type human SHBG for 2-MeOE2 is reduced in the presence of zinc chloride to approximately the same extent as that of estradiol (Fig. 2A). Furthermore, the negative effect of zinc binding on both of these C18 steroids was eliminated by substitution of His^{136} (Fig. 2B) and Asp^{65} (Fig. 2C) with glutamine and alanine, respectively, in order to prevent the coordination of a zinc ion at this site. Thus, the mechanism by which zinc reduces the binding affinity of estradiol is also operative for 2-MeOE2 and may have some interesting implications with respect to the possible use of zinc to enhance the pharmacological properties of 2-MeOE2 (12) by reducing its binding to SHBG.

Implications for the Binding of Other Estradiol C-2 Derivatives to Human SHBG—It has been noted previously (10) that human SHBG has an exceptionally high affinity for 2-io doestradiol exceeding that of DHT. When compared with 2-MeOE2, it is apparent that other derivatives of estradiol with halogen atoms (namely, fluorine or bromine) at the C-2 position exhibit higher affinities for human SHBG (Fig. 3). There may be several explanations for this. The functional group at C-2 of estradiol can be involved in direct hydrophilic interactions with SHBG, and the oxygen atom of the methoxy group of 2-MeOE2 appears to hydrogen-bond with the amido group of Asn^{42} in the SHBG crystal structure, as discussed above. With respect to the different C-2 derivatives of estradiol, the strength of this interaction should depend on the electronegativity of the hydrogen-bond acceptor at C-2. However, removal of the hydroxyl bond donor (amido group of Asn^{42}) by site-directed mutagenesis (N2A SHBG variant, Tables II and III) results in only moderate reductions in binding affinity for C-2 derivatives of estradiol.
 Binding of 2-Methoxyestradiol to Human SHBG

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1. Westphal, U. (1986) Steroid-Protein Interactions II. Monographs on Endocrinology, pp. 198–264, Springer-Verlag, Berlin
2. Hammond, G. L. (1995) Trends Endocrinol. Metab. 6, 298–304
3. Grishkovskaya, I., Avvakumov, G. V., Sklenar, G., Dales, D., Hammond, G. L., and Muller, Y. A. (2000) EMBO J. 19, 504–512
4. Avvakumov, G. V., Grishkovskaya, I., Muller, Y. A., and Hammond, G. L. (2001) J. Biol. Chem. 276, 33425–33435
5. Grishkovskaya, I., Avvakumov, G. V., Hammond, G. L., Catalano, M. G., and Muller, Y. A. (2002) J. Biol. Chem. 277, 32086–32090
6. Perto, C. S., Gunasal, G. L., Bardin, C. W., Phillips, D. M., and Musto, N. A. (1991) Endocrinology 129, 436–445
7. Avvakumov, G. V., Zhou, N. I., and Strelabhyunok, O. A. (1986) Biochim. Biophys. Acta 881, 488–495
8. Romer, W., Hryh, D. J., Khan, M. S., Nakha, A. M., and Romas, N. A. (1991) J. Steroid Biochem. Mol. Biol. 40, 813–820
9. Dunn, J. F., Niran, B. C., and Robard, D. B. (1981) J. Clin. Endocrinol. Metab. 53, 58–68
10. Fernlund, P., and Gehrsson, S. (1990) J. Steroid Biochem. 36, 75–81
11. D’Amato, R. J., Lin, C. M., Flynn, E., Folkman, J., and Hame, E. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 3964–3968
12. Pribulda, V. S., Gubish, E. R., Jr., LaVallee, T. M., Treston, A., Swartz, G. M., and Muller, Y. A. (2000) Trends Endocrinol. Metab. 11, 98–105
13. Grishkovskaya, I., Avvakumov, G. V., Sklenar, G., Dales, D., Beilhe, J., Hammond, G. L., and Muller, Y. A. (1999) Acta Crystallogr. Sect. D Biol. Crystallogr. 55, 2033–2035
14. Owczarksowski, M., and Mizor, W. (1997) Methods Enzymol. 276, 307–328
15. Fletcher, D. A., McMeeking, R. F., and Parkin, D. B. (1998) J. Chem. Inf. Comput. Sci. 38, 746–749
16. Kleywegt, G. J., and Jones, T. A. (1998) Acta Crystallogr. D Biol. Crystallogr. 54, 1119–1131
17. Brunger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J.-S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) Acta Crystallogr. Sect. D Biol. Crystallogr. 54, 905–921
18. Jones, T. A., Au, J. Y., Cowan, S. W., and Kjeldgaard, M. (1991) Acta Crystallogr. Sect. A 47, 101–110
19. Bocchino-Fuso, W. P., Warnels-Rodenhusen, S., and Hammond, G. L. (1991) Mol. Endocrinol. 5, 1723–1729
20. Hammond, G. L., and Lutkenmeaki, P. L. (1983) Clin. Chem. Acta 132, 101–110
21. Avvakumov, G. V., Muller, Y. A., and Hammond, G. L. (2000) J. Biol. Chem. 275, 25929–25935
22. Grishkovskaya, I., Avvakumov, G. V., Hammond, G. L., and Muller, Y. A. (2002) J. Mol. Biol. 318, 621–626
23. Rudenko, G., Hohenester, E., and Muller, Y. A. (2001) Trends Biochem. Sci. 26, 363–368
24. Wunderlich, V. H., and Mootz, D. (1971) Acta Crystallogr. Sect. B Struct. Sci. 27, 1684–1686
25. Pauling, L. C. (1960) The Nature of the Chemical Bond and the Structure of Molecules and Crystals: An Introduction to Modern Structural Chemistry, 3rd Ed., p. 494, Cornell University Press, Ithaca, NY
26. Cafferata, E. L., Stack, D. E., DeLano, W. L., Todrovic, R., Devivo, J., Hegghebhandh, S. Johannes, S. L., Patil, K. D., Gros, M. L., Gooden, J. K., Ramanathan, R., Cerny, R. L., and Rogen, E. G. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 10987–10982
27. Dawling, S., Rood, N., Mennough, R. L., Wang, X., and Parle, F. F. (2001) Cancer Res. 61, 6716–6722
28. Brueggemeier, R. W., Bhat, A. S., Lovely, C. J., Coughounour, H. D., Joopchabutra, S., Witzel, D. H., Vander, D. D., Yusuf, F., and Burak, W. E., Jr. (2001) J. Steroid Biochem. Mol. Biol. 78, 145–156
29. Seimbille, Y., Rousseau, J., Bénard, F., Morin, C., Ali, H., Avvakumov, G., Hammond, G. L., and van Lier, J. E. (2002) Steroids 67, 765–775
30. Krailis, P. J. (1991) J. Appl. Crystallogr. 24, 946–950
31. Merritt, E. A., and Murphy, M. E. F. (1994) Acta Crystallogr. Sect. D Biol. Crystallogr. 50, 869–877
32. Verbiest, J. J., Lehman, M. S., Keetle, T. F., and Hamilton, W. C. (1972) Acta Crystallogr. Sect. B Struct. Sci. 28, 3006–3013

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REFERENCES
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