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The sex steroid binding protein (SBP or SHBG) of human plasma: identification of Tyr-57 and Met-107 in the steroid binding site

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Abstract

Tyrosine-57 (Y57) and methionine-107 (M107) have been identified in the binding site of the sex steroid binding protein (SBP) (or sex hormone binding globulin) of human plasma by replacing the two amino acids with a number of residues of varying structure. Replacement of Y57 with phenylalanine resulted in a fourfold increase in the K_d of 5α-dihydrotestosterone but left the K_d of 17β-estradiol unchanged. Except in two cases, no further loss in binding took place when replacing Y57 with other residues, suggesting that the phenolic group of Y57 may form a hydrogen bond with the ligand. Replacement of M107 with isoleucine increased the 5α-dihydrotestosterone K_d fourfold to a value equal to that of rabbit SBP, which contains isoleucine at the corresponding position; however, the K_d of 17β-estradiol remained unchanged. Replacement of M107 with threonine resulted in a tenfold decrease in 5α-dihydrotestosterone binding affinity, whereas replacement with leucine left the K_d unchanged. These data indicate that substitutions on the β-carbon of the amino acid side-chain at position 107 causes significant loss of binding affinity but, as in the case of Y57, the activity was not totally eliminated. We conclude that Y57 and M107 form part of a structural motif within the steroid binding site and specifically contribute binding energy to ring A of 5α-dihydrotestosterone but not to ring A of 17β-estradiol. We also propose that the integrated contribution of several side chains may be required to optimize the ligand affinity of the steroid binding site. This proposal may fit a 'lock and key' model where little movement of the side chains occurs during binding as might be expected for a rigid structure like the steroid nucleus.

Keywords: Sex steroid-binding protein; Sex hormone binding globulin; Dihydrotestosterone; Testosterone; 17β-Estradiol

1. Introduction

Human sex steroid-binding protein (SBP) (also referred to as sex hormone binding globulin) is a 93.4 kDa homodimeric glycoprotein secreted by the liver that specifically binds testosterone (T), dihydrotestosterone (DHT), and 17β-estradiol (E_2) with high affinity in human plasma [1–3]. The physiological role of SBP is to regulate the bioavailability of testosterone and 17β-estradiol in plasma by controlling their metabolic clearance rates [4–7]. SBP has also been proposed to play additional roles in the uptake of hormone by cells [8–10] and in signaling [11,12]. Human and rabbit SBPs are 80% identical in their amino acid sequences [13–15] and they both bind 2 mol of calcium per monomer [16]. The metal does not appear to play a role in steroid

Abbreviations: SBP, plasma sex steroid-binding protein; DHT, 5α-dihydrotestosterone; T, testosterone; E2, 17β-estradiol; Y57, tyrosine-57; M107, methionine-107; M139, methionine-139; I101, isoleucine-101; K134, lysine-134; Y57F, replacement of Y57 by phenylalanine at position 57; Y57A, replacement of Y57 by alanine at position 57; Y57T, replacement of Y57 by threonine at position 57; Y57G, replacement of Y57 by glycine at position 57; Y57L, replacement of Y57 by leucine at position 57; Y57S, replacement of Y57 by serine at position 57; M107I, replacement of M107 by isoleucine at position 107; M107L, replacement of M107 by leucine at position 107; M107T, replacement of M107 by threonine at position 107; M107A, replacement of M107 by alanine at position 107.

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binding or dimerization [17] but it could function in stabilizing the folding of certain regions of the protein, as indicated by the protection of calcium against heat denaturation [18]. Electron microscopy indicates that SBP has a rod-like structure, and circular dichroism studies reveal that the protein contains 15% helix, 43% β-sheet, and 10–16% β-turns [19]. Human SBP has a low degree of similarity with the amino acid sequences of the C-terminal domains of the vitamin-K-dependent protein S [20,21] and of domain G of the laminin α chains [22,23]. Although none of these proteins bind the sex steroids, the C-terminus G domain of the latter protein appears to be structurally related to SBP [19]. Human and rabbit SBPs differ in their steroid binding specificities: the former binds both DHT and E_2, while the latter has a threefold lower binding affinity for DHT and a 20-fold lower binding affinity for E_2. It thus appears that the steroid binding site of the human protein is larger than that of the rabbit protein so that it can accommodate both steroid hormones. To understand the molecular basis of steroid binding specificity, this laboratory is interested in identifying amino acid side-chains in the steroid binding site of SBP. To this end, Met-139 and Lys-134 have been identified in or near the steroid binding site by chemical modification and site-directed mutagenesis [24–26]. We now report two additional residues, tyrosine-57 (Y57) and methionine-107 (M107), that function in ligand binding.

2. Materials and methods

2.1. Materials

pcDNA3 was purchased from Invitrogen (San Diego, CA). QuickChange™ site-directed mutagenesis kits and Epicurian Coli supercompetent cells were purchased from Stratagene. The ampliCycle sequencing kit was purchased from Perkin Elmer. DHT, T, and E_2 were purchased from Steraloids (Wilton, NH). DMEM, calf serum, AIM V Media, Lipofectamine 2000 reagent, and custom primers were ordered from Life Technologies. [1,2-^{3}H]DHT (58.4 Ci/mmol) and [6,7-^{3}H]E_2 (48 Ci/mmol) were purchased from New England Nuclear (Boston, MA). Enzyme-linked immunosorbent assay (ELISA) kits for SBP determinations in media were provided by RADIM (Angleur, Belgium), and the rabbit anti-goat IgG coupled to alkaline phosphatase used in Western blots was bought from Bio-Rad (Hercules, CA, USA). All other equipment and chemicals used in this work are described in cited publications from this laboratory.

2.2. DNA constructions, site-directed mutagenesis, and SBP expression

Full-length wild-type SBP cDNA [27] was subcloned in pcDNA3. Site-directed mutagenesis was carried out with the QuickChange™ site-directed kit (Stratagene) using *Pfu* DNA polymerase with sense and antisense oligonucleotide mutated primers. The wild-type template strand was digested with *Dpn* I, which targets the sequence 5′-G^{m6}ATC-3′ [28]. The remaining nicked plasmid was transformed into competent *Escherichia coli*, and colonies were isolated for DNA purification and sequencing. Transfections were carried in COS-7 cells with the Lipofectamine 2000 reagent as described by the manufacturer. Transfected cells were grown in serum-free AIM V medium, and SBP expression was determined by ELISA and ligand binding assays.

2.3. Measurement of steroid binding activity

The total concentration of SBP in transfection media was determined by the charcoal assay with [1,2-^{3}H]DHT [25]. Equilibrium constants of DHT dissociation were determined directly by Scatchard analyses; equilibrium dissociation of E_2 was determined either directly with [6,7-^{3}H]E_2 or by competitive Scatchard analyses [29] using 1–2 nM SBP samples and a range of 1–10 nM [1,2-^{3}H]DHT in the presence or absence of 8 nM radioinert E_2 for human SBP and 25 nM radioinert E_2 for rabbit SBP. The samples were incubated for 30 min at 25°C, cooled to 4°C, and treated with charcoal. For direct Scatchard analyses, 1–2 nM SBP samples were incubated with 1–10 nM [^{3}H]DHT or with 1–12 nM [6,7-^{3}H]E_2. For SBP mutants with K_d values for DHT higher than the wild type, the K_d value for E_2 was determined directly with [6,7-^{3}H]E_2 because the competitive Scatchard method leads to an apparent higher value.

3. Results

3.1. Effects of replacing tyrosine-57 with other amino acid residues

To investigate the possible function of the sole tyrosine residue in steroid binding, Y57 was replaced with seven different amino acid residues of varying structure. The resulting effects on steroid binding affinity were determined after the mutated cDNAs were expressed in COS-7 cells as previously described [25]. In the present instance, however, following transfection, the cells were transferred to serum-free medium to remove bovine SBP in calf serum instead of correcting for its presence. The results, shown in Table 1 and Fig. 1, indicate that eliminating the phenolic group of Y57 by replacing

with phenylalanine produced a 3.9-fold ($\pm$ 0.4) increase in the K_d of DHT, while leaving the E_2 binding affinity little changed. Additionally, replacement of Y57 with smaller non-aromatic hydrophobic or hydrophilic residues, such as the Y57L and Y57S mutants (Table 1), resulted in little additional loss of DHT binding affinity but left the estradiol binding affinity unchanged. The two exceptions were the Y57T and Y57G mutants, those were totally inactive in binding DHT and E_2 but their transfection media were ELISA-positive, suggesting that SBP was expressed and appeared to fold normally.

3.2. Effects of replacing methionine-107 with other amino acid residues

Because methionine-139 (M139) was previously identified in the steroid binding site of human SBP [24,25], M107 located near M139 [13] was tested as a possible residue contributing energy of binding to DHT. M107 was mutated to other residues, and the results are shown in Table 2 and in Fig. 2. Replacement of M107 with isoleucine lowered DHT binding affinity about fourfold while leaving estradiol binding affinity relatively unchanged, as in the case of the Y57 mutations. Interestingly, the K_d value of the M107I mutant (1.75 nM) is identical to that of native rabbit protein, which also contains an isoleucine at the corresponding position (I101) [14,15]. Replacement of M107 with threonine, another amino acid containing a substituent on the β-carbon, lowered the DHT binding affinity tenfold when compared with the wild type (Table 2); however, the DHT K_d remained unchanged when M107 was replaced with leucine, and increased slightly when replaced with alanine.

Table 1
Equilibrium constants of Y57 mutants of human SBP expressed in COS-7 cells at 4°C (pH 7.5)

Mutation	K_d(DHT)[a] (nM)	K_d(E_2) (nM)
Human plasma SBP	0.42[b]	4.79[c], 4.39[d]
WT human SBP (Y57)	0.44	3.83[c]
Y57F[e]	2.00[f], 1.45	3.75[d]
Y57M	2.11	5.30[d]
Y57L	3.54	4.54[d]
Y57A	4.12	5.32[d]
Y57S	2.54	3.15[d]
Y57G	Inactive[g]	Inactive[g]
Y57T	Inactive[g]	Inactive[g]

[a] Calculated by direct Scatchard analysis using 1–10 nM [^{3}H]DHT and 1–2 nM SBP. Average of two or three determinations.
[b] Mickelson and Petra [29].
[c] Calculated by competitive Scatchard analysis [29] using 1–10 nM [^{3}H]DHT, 8 nM radioinert E_2, and 1–2 nM SBP.
[d] Calculated by direct Scatchard analysis using 1–12 nM [^{3}H]E_2 and 1–2 nM SBP.
[e] See Fig. 1.
[f] Sui et al. [25].
[g] ELISA-positive.

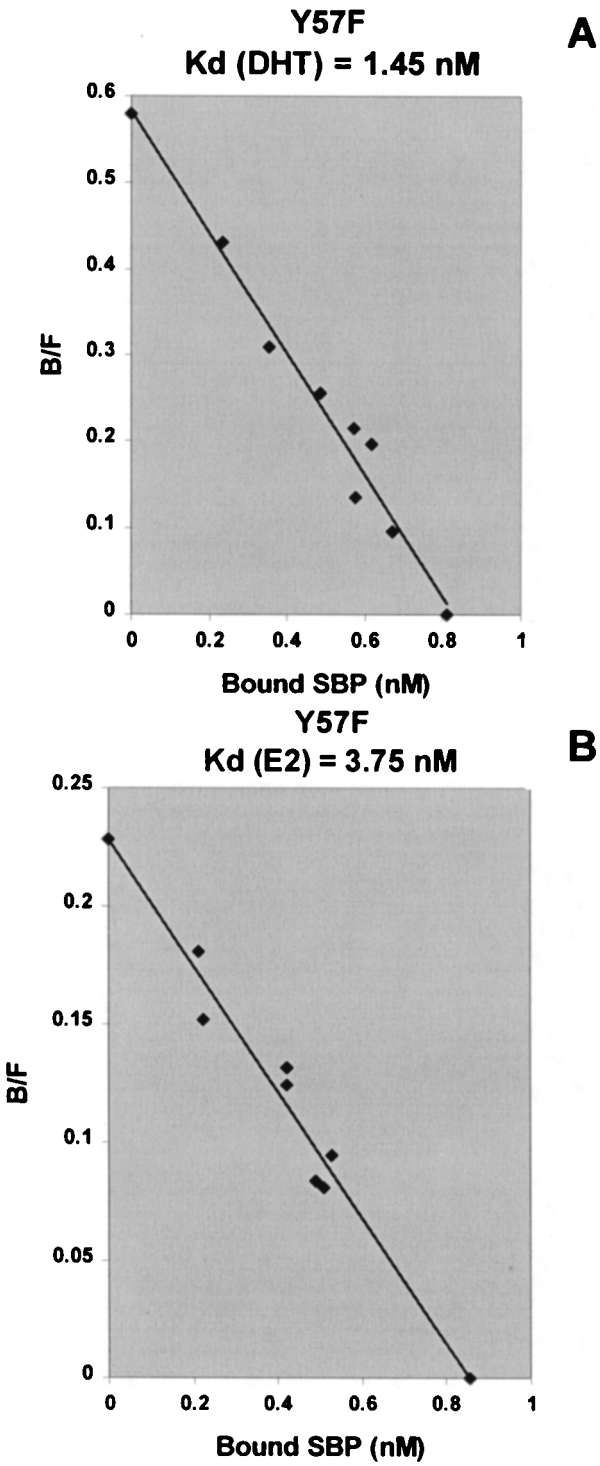

Fig. 1. (A) Determination of the equilibrium constant of 5α-dihydrotestosterone dissociation of the Y57F human SBP mutant at pH 7.4 and 4°C. [^{3}H]DHT concentration, 1–10 nM; SBP concentration, 1 nM. (B) Determination of the equilibrium constant of 17β-estradiol dissociation of the Y57F human SBP mutant at pH 7.4 and 4°C. [^{3}H]E_2 concentration, 1–8 nM; SBP concentration, 2 nM.

4. Discussion

One of the objectives of this laboratory is to identify functional amino acid residues in the steroid binding site of SBP to define ligand-binding specificity. The approach has been affinity labeling and site-directed mutagenesis. Expression of SBP in yeast [30] has allowed purification of the full-length deglycosylated protein dimer to initiate crystallization trials (experiments in progress), which should allow functional studies to be complemented with structural information. Because SBP is the only protein in human plasma that specifically binds testosterone and 17β-estradiol, mapping the steroid binding site functionally and structurally in three dimensions could be useful for designing steroidal drugs that might be effective in the treatment of osteoporosis and breast and prostate cancer. Recent published work on the structure of the non-homologous E_2 binding domains of 17β-hydroxysteroid dehydrogenase [31] and of the estrogen receptor [32] reveals numerous van der Waal interactions between their steroid ligand and the protein surface. Because the steroid binding affinities of these proteins are comparable with SBP, it seems likely that other amino acid residues, in addition to M139 and lysine-134 (K134), will contribute binding energy to the ligand in that protein. We now report that Y57 and M107 are important for DHT binding but, surprisingly, play no role in the binding of E_2. Because the difference between these two steroids resides primarily in the structure and orientation of ring A of the steroid, these data suggest that Y57 and M107 are located near each other and in close proximity to the saturated ring A of DHT. The findings further indicate that Y57 and M107 are within the steroid-binding site because, if they were located elsewhere, replacement with other residues would have produced a loss of both DHT and E_2 binding affinity, presumably through a protein conformation change.

Table 2
Equilibrium constants of M107 mutants of human SBP expressed in COS-7 cells at 4°C (pH 7.5)

Mutation	K_d(DHT)[a] (nM)	K_d(E_2) (nM)
Human plasma SBP	0.42[b]	4.79[c], 4.39[d]
WT human SBP (M107)	0.44	3.83[c]
Rabbit plasma SBP	1.77[e]	84.6[b], 83.31[f]
M107I[g]	1.75	2.92[d]
M107L	0.32	4.06[c]
M107T	4.52	–
M107A	0.77	6.19[d]

[a] Calculated by direct Scatchard analysis using 1–9 nM [^{3}H]DHT and 1–2 nM SBP. Average of two or three determinations.
[b] Mickelson and Petra [29].
[c] Calculated by competitive Scatchard analysis using 1–9 nM [^{3}H]DHT, 8 nM radioinert E_2, and 1–2 nM SBP.
[d] Calculated by direct Scatchard analysis using 1–12 nM [^{3}H]E_2 and 1–2 nM SBP.
[e] The previously reported K_d DHT value of 0.86 nM for rabbit SBP [29] was found to be too low. This re-determined value represents an average of three measurements.
[f] Re-evaluated as calculated by competitive Scatchard analysis using 1 –9 nM [^{3}H]DHT, 25 nM radioinert E_2, and 1–2 nM SBP.
[g] See Fig. 2.

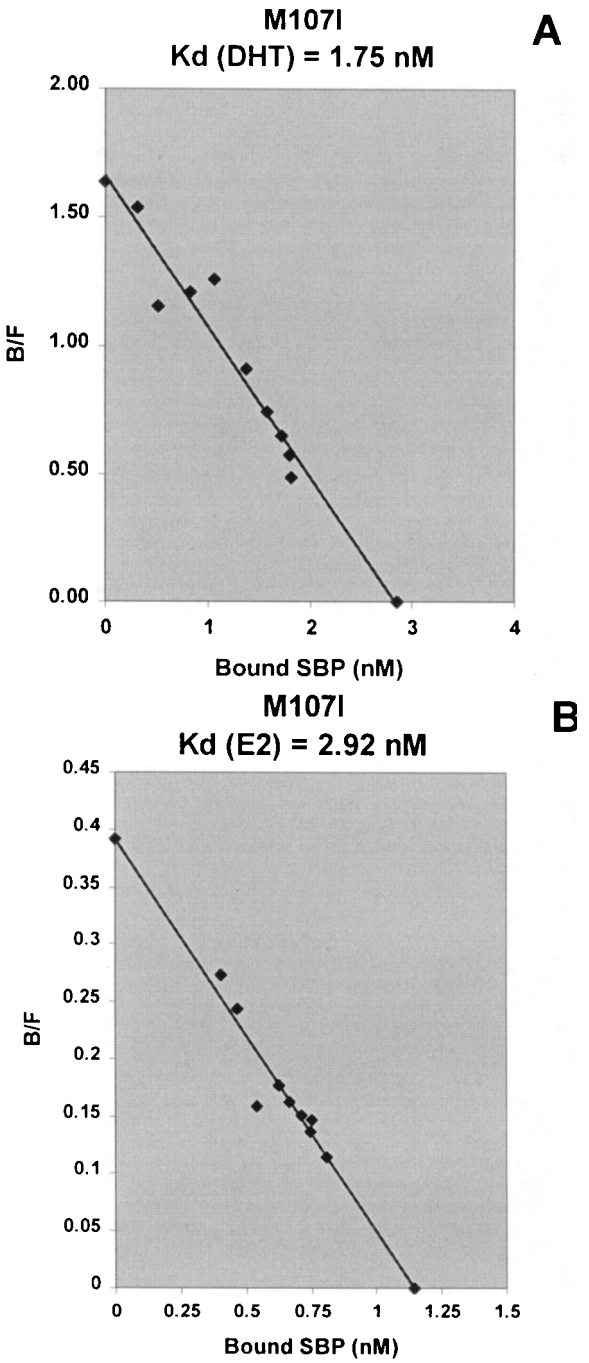

Fig. 2. (A) Determination of the equilibrium constant of 5α-dihydrotestosterone dissociation of the M107I human SBP mutant at pH 7.4 and 4°C. [^{3}H]DHT concentration, 1–10 nM; SBP concentration, 2 nM. (B) Determination of the equilibrium constant of 17β-estradiol dissociation of the M107I human SBP mutant at pH 7.4 and 4°C. [^{3}H]E_2 concentration, 1–8 nM; SBP concentration, 2 nM.

In the case of Y57, the major loss in DHT binding affinity occurred when it was replaced with phenylalanine; no further reduction was observed when it was replaced with other amino acid residues, except in the case of the Y57G and Y57T mutants. Although the Y57G and Y57T mutants were inactive under ligand saturation conditions, their transfection media were ELISA-positive. This finding suggests that replacement of tyrosine with threonine or glycine results in the collapse of the steroid binding site without affecting the normal folding of the protein and its expression. Taken together, these results suggest that the phenolic group of Y57 forms a hydrogen bond either with the 3′-keto group of ring A of the steroid, as proposed earlier by fluorescence quenching experiments on human and rabbit SBP [33], or with another group close to the steroid binding site. On the other hand, methionine-107 is more likely to stabilize binding through hydrophobic interactions with the steroid ligand because its replacement with less hydrophobic amino acids decreased the steroid binding affinity. Moreover, the DHT binding affinity is four- to ten-fold lower when M107 is replaced with isoleucine and threonine, respectively, but not with leucine and alanine, which essentially yield normal K_d values (Table 2). This means that a substituent on the β-carbon of the amino acid side-chain at position 107 lowers the DHT binding affinity significantly. Because isoleucine is the wild-type amino acid at the corresponding position in rabbit SBP (I101), this finding suggests that placing a substituent on the β-carbon may have been the step responsible for lowering DHT binding affinity in the evolution of the rabbit SBP DHT binding site. In addition, since the rabbit protein does not bind E_2 significantly, other replacements have occurred during the divergent evolution of these two homologous proteins to account for the difference in 17β-estradiol binding specificity. In this context, we have recently identified the amino acid residues responsible for that difference (manuscript in preparation).

By determining the DHT and E_2 binding affinities of a hybrid dimer composed of one human SBP subunit and one rabbit SBP subunit, we have shown that the region binding ring A of DHT differs from that binding ring A of E_2 [17]. We propose that the side chains of Y57 and M107 reside within the structural motif that binds ring A of DHT because replacing them with other residues reduces DHT binding affinity but leaves E_2 binding affinity unchanged. Moreover, because the M107I human SBP mutant binds DHT with the same K_d value (1.75 nM) as native rabbit SBP (1.77 nM), we also propose that the corresponding isoleucine in rabbit SBP, I101, is similarly located. By analogy, the sole tyrosine at position 51 in rabbit SBP is probably located in the steroid binding site.

Except for the Y57G and Y57T mutants, each replacement or modification at positions 57, 107, and 134 [26] results in only a partial reduction of steroid binding affinity. These data suggest that the SBP steroid binding site may be structurally rigid, because modification of any single side chain reduces the affinity by only a small factor, and apparently leaves the other interactions undisturbed. This reasoning leads to the conclusion that the integrated contribution of most of the side chains in the binding site is required to optimize ligand-binding affinity. As proposed for protein binding sites by Emil Fischer [34], we suggest that the steroid binding site may fit a 'lock and key' model in which little movement of the side chains occurs during ligand binding, and the unbound site is similar in structure to the bound site. This structural design may be particularly suitable for binding a rigid structure like the steroid nucleus. In contrast, replacing M139 with amino acids having decreased hydrophobicity results in a complete loss of steroid binding activity [25], suggesting that M139 plays a critical role in high binding affinity. It is not clear whether this complete loss of binding activity is caused by a loss in hydrophobicity or by the structural collapse of the steroid binding site, as proposed for Y57G and Y57T. Finally, because human SBP is a dimer of identical subunits [35], the way in which each subunit contributes to the formation of the single steroid binding site is an open question. We have presented evidence that the binding site may be located at the interface between the subunits [17] where amino acid side chains from each subunit would be expected to contribute to ligand binding. Although we believe that M139 and K134 are likely to reside within the same subunit because of their close proximity in the linear sequence, Y57 and M107 are far apart and could be located in either subunit. It should be possible to clarify this issue through X-ray diffraction analyses, and chemical modifications and site-directed mutation of the human SBP/rabbit SBP hybrid.

In summary, we have presented evidence for the presence of two amino acid residues, Y57 and M107, in addition to K134 and M139 in the steroid binding site of human SBP. The data indicate that the two side chains provide binding energy for DHT but play no role in E_2 binding. It is suggested that the two side chains are in close proximity and form part of the structural motif that recognizes the ring A of the two androgens T and DHT.

Note added in proof — After submission of this manuscript, we noted the publication of a crystal structure of the N-terminal domain (residues 1 to 205) of human SBP (Grishkovskaya et al., EMBO 19:(2000)504). The crystal structure data are generally

consistent with those presented here. More details on the comparison will appear in a forthcoming paper from this laboratory.

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References

[1] U. Westphal, Steroid–Protein Interaction II. In: Monograph on endocrinology, vol. 27, Springer-Verlag, New York, 1986.
[2] P.H. Petra, The plasma sex steroid-binding protein (SBP or SHBG). A critical review of recent developments on the structure, molecular biology, and function, J. Steroid Biochem. Mol. Biol. 40 (1991) 735–753.
[3] D.R. Joseph, Structure, function, and regulation of androgen-binding protein/sex hormone-binding globulin, Vitamins Hormones 49 (1994) 197–280.
[4] C.W. Bardin, M.B. Lipsett, Testosterone and androstenedione blood production rates in normal women and women with idiopathic hirsutism or polycystic ovaries, J. Clin. Invest. 46 (1967) 891–902.
[5] A.L. Vermeulen, L. Verdonck, M. Van der Straeten, M. Orie, Capacity of the testosterone binding globulin in human plasma and influence of specific binding of testosterone on its metabolic clearance rate, J. Clin. Endocrinol. Metab. 29 (1969) 1470–1480.
[6] P.H. Petra, F.Z. Stanczyk, P.C. Namkung, M.A. Fritz, M.J.: Novy, Direct influence of the sex steroid-binding protein (SBP) of plasma on the metabolic clearance rate of testosterone, J. Steroid Biochem. 22 (1985) 739–746.
[7] S.R. Plymate, P.C. Namkung, L.A. Matej, P.H. Petra, Direct effect of plasma sex hormone binding globulin (SHBG) on the metabolic clearance rate of 17β-estradiol in the primate, J. Steroid Biochem. 36 (1990) 311–317.
[8] S. Bordin, P.H. Petra, Immunocytochemical localization of the plasma sex steroid-binding protein (SBP) in tissues of the adult male monkey, *Macaca nemestrina*, Pro. Natl. Acad. Sci. USA 77 (1980) 5678–5682.
[9] O.A. Strel'chyonok, G.V. Avvakumov, L.I. Survilo, A recognition system for sex-hormone-binding protein–estradiol complex in human decidual endometrium plasma membranes, Biochem. Biophys. Acta 802 (1984) 459–466.
[10] W.M. Pardridge, Selective delivery of sex steroid hormones to tissues in vivo by albumin and sex hormone-binding globulin, Ann. NY Acad. Sci. 538 (1988) 173–192.
[11] A.M. Nakhla, M.S. Khan, W. Rosner, Biologically active steroids activate receptor-bound human sex hormone-binding globulin to cause LNCaP cells to accumulate adenosine 3′,5′-monophosphate, J. Clin. Endocrinol. Metab. 71 (1990) 398–404.
[12] F. Fissore, N. Fortunati, A. Comba, A. Fazzari, G. Gaidano, L. Berta, R. Frairia, The receptor-mediated action of sex steroid-binding protein (SBP, SHBG): accumulation of cAMP in MCF-7 cells under SBP and estradiol treatment, Steroids 59 (1994) 661–667.
[13] K.A. Walsh, K. Titani, S. Kumar, R. Hayes, P.H. Petra, Amino acid sequence of the sex steroid-binding protein (SBP) of human blood plasma, Biochemistry 25 (1986) 7584–7590.
[14] P.R. Griffin, S. Kumar, J. Shabanowitz, H. Charbonneau, P.C. Namkung, K.A. Walsh, D.F. Hunt, P.H. Petra, The amino acid sequence of the sex steroid-binding protein of rabbit serum, J. Biol. Chem. 264 (1989) 19066–19075.
[15] W.M. Lee, A.S.T. Wong, C.-H. Cheung, J.C.H. Li, G.L. Hammond, Rabbit sex hormone binding globulin: primary structure, tissue expression, and structure/function analyses by expression in *Escherichia coli*, J. Endocrinol. 153 (1997) 373–384.
[16] J.B.A. Ross, P.B. Contino, M.F. Lulka, P.H. Petra, Observation and quantitation of metal binding sites in the sex steroid-binding protein of human and rabbit sera using the luminescent probe, terbium, J. Protein Chem. 4 (1985) 299–304.
[17] L.M. Sui, W. Hughes, A. Hoppe, P.H. Petra, Direct evidence for the localization of the steroid-binding site of the plasma sex steroid-binding protein (SBP or SHBG) at the interface between the subunits, Protein Sci. 5 (1996) 2514–2522.
[18] W. Rosner, S. Toppel, R.N. Smith, Testosterone–estradiol-binding globulin of human plasma: denaturation and protection, Biochem. Biophys. Acta 351 (1974) 92–98.
[19] K. Beck, T. Gruber, C.C. Ridgway, W. Hughes, L.M. Sui, P.H. Petra, Secondary structure and shape of plasma sex steroid-binding protein. Comparison with domain G of laminin results in a structural model of plasma sex steroid-binding protein, Eur. J. Biochem. 247 (1997) 339–347.
[20] M.E. Baker, F.S. French, D.R. Joseph, Vitamin K-dependent protein S is similar to rat androgen-binding protein, Biochem. J. 243 (1987) 293–296.
[21] S. Gershagen, P. Fernlund, A. Lundwall, A cDNA coding for human sex hormone binding globulin. Homology to vitamin K-dependent protein S, FEBS Lett. 220 (1987) 129–135.
[22] K. Beck, J. Spring, R. Chiquet-Ehrismann, J. Engel, M. Chiquet, Structural motifs of the extracellular matrix proteins laminin and tenascin, Springer Ser. Biophys. 7 (1992) 229–254.
[23] D.R. Joseph, M.E. Baker, Sex hormone binding globulin, androgen-binding protein, and vitamin K-dependent protein S are homologous to laminin A, merosin, and Drosophila crumbs protein, FASEB J. 6 (1992) 2477–2481.
[24] C. Grenot, A. Montard, T. Blachere, M. Rolland de Ravel, E. Mappus, C.Y. Cuilleron, Characterization of Met-139 as the photolabeled amino acid residue in the steroid binding site of sex hormone binding globulin using Δ6 derivatives of either testosterone or estradiol as unsubstituted photoaffinity labeling reagents, Biochemistry 31 (1992) 7609–7621.
[25] L.M. Sui, A.W.C. Cheung, P.C. Namkung, P.H. Petra, Localization of the steroid-binding site of the human sex steroid-binding protein of plasma (SBP or SHBG) by site-directed mutagenesis, FEBS Lett. 310 (1992) 115–118.
[26] P.C. Namkung, S. Kumar, K.A. Walsh, P.H. Petra, Identification of Lysine-134 in the steroid binding site of the sex steroid binding protein of human plasma, J. Biol. Chem. 265 (1990) 18345–18350.
[27] F.S. Hagen, C. Arguelles, L.-M. Sui, W. Zhang, P.R. Seidel, S.C. Conroy, P.H. Petra, Mammalian expression of the human sex steroid-binding protein of plasma (SBP or SHBG) and testis (ABP). Characterization of the recombinant protein, FEBS Lett. 299 (1992) 23–37.
[28] M. Nelson, M. McClelland, Methods Enzymol. 216 (1992) 279–303.
[29] K.E. Mickelson, P.H. Petra, Purification and physico-chemical characterization of the sex steroid-binding protein of rabbit serum. Comparison with the human protein, J. Biol. Chem. 253 (1978) 5293–5298.
[30] L.M. Sui, J. Lennon, C. Ma, I. McCann, I. Woo, P.H. Pétra, Heterologous expression of wild type and deglycosylated hu-

man plasma sex steroid-binding protein (SBP or SHBG) in the yeast, *Pichia pastoris*. Characterization of the recombinant proteins, J. Steroid Biochem. Mol. Biol. 68 (1999) 119–127.

[31] A. Azzi, P.H. Rehse, D. Zhu, R.L. Campbell, F. Labrie, S.-X. Lin, Crystal structure of human estrogenic 17β-hydroxysteroid dehydrogenase complexed with 17β-estradiol, Nat. Struct. Biol. 3 (1996) 665–668.

[32] A.M. Brzozowski, A.C.W. Pike, Z. Dauter, R.E. Hubbard, T. Bonn, L. Engstrom, G.J. Greene, J.-A. Gustafsson, M. Carlquist, Molecular basis of agonism and antagonism in the oestrogen receptor, Nature 398 (1997) 753–758.

[33] A. Orstan, M.F. Lulka, B. Eide, P.H. Petra, J.B.A. Ross, Steroid-binding site of human and rabbit sex steroid-binding protein of plasma: fluorescence characterization with equilinin, Biochemistry 25 (1986) 2686–2692.

[34] E. Fischer, Einfluss der configuration auf die Wirkung der Enzyme, Ber. Deut. Chem. Ges. 27 (1894) 2985.

[35] P.H. Petra, S. Kumar, R. Hayes, L.H. Ericsson, K. Titani, Molecular organization of the sex steroid-binding protein (SBP) of human plasma, J. Steroid. Biochem. 24 (1986) 45–49.