5. Summary

Human sex hormone binding globulin (SHBG) transports sex steroids in blood and regulates their access to target tissues. In biological fluids, SHBG exists as a homodimer and each monomer comprises two laminin G-like domains (LG domains). The crystal structure of the amino-terminal LG domain of SHBG in complex with 5α-dihydrotestosterone at 1.55 Å resolution reveals the structure of the LG domain as such as well as the architecture of the steroid-binding site and the quaternary structure of the dimer. It was shown that LG domains have jellyroll topology and are structurally related to pentraxin. LG domains appear to have a unique multifunctional ligand-binding site distinct from carbohydrate-binding sites found in lectins.

Analysis of the human SHBG crystal structure indicated that homodimerization occurs through a two-fold symmetry axis located at the edge of the β-sheet sandwich of each SHBG monomer. This symmetry axis places strand β7 of one monomer next to strand β10 of the other monomer and vice versa. As a consequence, the main-chain hydrogen bond pairing characteristic for β-sheets extends across the interface, and two contiguous 14-stranded β-sheets are generated in the SHBG homodimer. In each SHBG monomer, the steroid intercalates into a hydrophobic pocket within the β-sheet sandwich. The steroid and a 20 Å distant calcium ion are not located at the dimer interface. Instead, two separate steroid binding pockets and calcium binding sites exist per dimer. The structure displays disorder for the loop segment Pro130 to Arg135.

The crystal structures of the tetragonal crystal form and the EDTA-soaked trigonal crystals of SHBG completed the original structure and reveal the loop segment that covers the steroid-binding pocket. Thus, a more detailed description of the steroid-binding site topography is obtained. In both of these structures, residues Pro130 to Arg135 are clearly visible. A 3_{10} helical turn is formed by residues Leu131 to Lys134 in this segment. Unfolding of this secondary structure element presumably facilitates the entry of the steroids into the binding site or modulate the important contribution that Leu131 makes to steroid binding. The binding of zinc reorients the side-chain of His136 as observed in the original crystal structure

of SHBG and in the zinc- complexed crystal structure. Apparently, this residue causes disorder within the loop structure between Pro130 and Arg135.

The crystal structures of SHBG in complex with different ligands, which are estradiol, 5α-androstane, 3β,17β-diol (17β–DHA), 5α-androstane, 3β,17α-diol (17α–DHA), 2-methoxyestradiol (methoxyestradiol), norgestrel, have also been solved. At a first glance, the adaptibility of the steroid-binding site in SHBG would be predicted to be rather limited. The steroid tightly intercalates in-between the β-sheet sandwich and is tightly packed between numerous hydrophobic residues lining the binding site. However, different SHBG-steroid complexes revealed that the steroid-binding pocket of SHBG is very adaptable and displays different possibilities to accommodate the ligands. The binding may occur in two ways, in a "forward" mode like DHT and other chemically related androgens or in the "reverse" mode like in estradiol and it metabolite methoxyestradiol. Depending on which ligand is bound, conformational rearrangements of the residues lining the pocket occur. For example, the position of Leu171 shifts outwards and the position of Leu131 shifts inwards in direction of the methyl group of estradiol. The hydroxyl group at the C3 atoms of 17β- and 17α-hydroxy-DHA is accommodated by a peptide flip. The X-ray structures of SHBG-steroid complexes presented here provide a three-dimensional composite of key molecular features for the binding of androgens and estrogens.