Crystal structure of human sex hormone binding globulin

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Sex hormone-binding globulin (SHBG) is produced in the liver and circulates in blood plasma as the major sex-steroid carrier protein. Human SHBG is a Ca^{2+}-dependent homodimeric glycoprotein which binds androgens and estrogens with nanomolar affinities. The 373 residue-long monomer consists of a repeat of two laminin G-like domains (G domains). Truncation experiments showed that the N-terminal G domain is sufficient for steroid binding and formation of homodimers [1]. Apart from laminin and SHBG, G domains are found in a variety of proteins such as blood coagulation factor protein S, growth arrest-specific protein 6 (GAS6), crumbs and many others. Until recently, no structural information was available on the tertiary fold of these domains.

The N-terminal G domain of SHBG (Residues 1 to 205) in complex with 5-α-dihydrotestosterone crystallized in two different crystal forms. Whereas the tetragonal crystals ($P4_122$, a = 52.2 Å, b = 148.4 Å) diffract to only 3.0 Å, the trigonal crystals (R32, a = 104.0 Å, b = 84.4 Å) diffract better than 1.6 Å resolution [2]. The structure was solved in the trigonal space group by the MIRAS method making use of a $PtCl_4$ derivative collected at beamline X31 of the EMBL outstation at DESY at a wavelength of 1.072 Å. Combining this data with additional heavy atom derivatives collected with Cu-Kα radiation yielded an experimental electron density map which was readily interpretable. A 1.55 Å native data set (Completeness = 94,6 %, R_{Merge} = 4.2 %) collected at beamline X11 allowed for the automatic extension of the phases with the ARP/WARP program package. Automatic chain tracing and subsequent sequence docking resulted in a model consisting of 122 out of 205 residues. Of those, 52 % were built according to their correct sequence while the remaining were modeled as alanines or serines. The model was then manually corrected and subjected to several cycles of refinement with the program REFMAC. The final model has a crystallographic R-Factor of 20.5 % (R-Free = 25.1 %).

The structure of the N-terminal domain of SHBG reveals a number of interesting features with respect to both the biological function of SHBG and the entire family of G domain-containing proteins [3]. The structure shows that G domains have jellyroll topology and are structurally related to pentraxin. In each SHBG monomer, the steroid intercalates into a hydrophobic pocket within the β-sheet sandwich. Unexpectedly, the steroid and a 20 Å distant calcium ion are not located in the dimer interface. Instead, two separate steroid binding pockets and calcium binding sites exist per dimer. This contradicts previous estimates of the steroid-binding ratio. The structure displays intriguing disorder for loop segment Pro 130 to Arg 135. In all other jellyroll proteins, this loop is well ordered and if modeled accordingly would cover the steroid binding site. We propose a model in which this loop regulates access of ligands to the binding pocket.

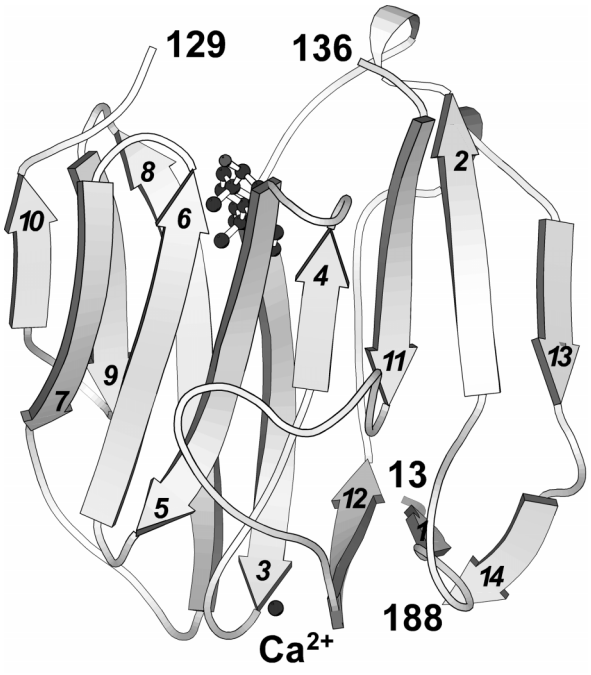

Figure 1: Ribbon representation of the N-terminal laminin G-like domain of SHBG

References

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