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Review

Molecular and structural basis of steroid hormone binding and release from corticosteroid-binding globulin

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ABSTRACT

Corticosteroid-binding globulin (CBG), a non-inhibitory member of the serine proteinase inhibitor (serpin) super-family, is the high-affinity transport protein for glucocorticoids in vertebrate blood. Plasma CBG is a glycoprotein with 30% of its mass represented by N-linked oligosaccharide chains. Its well-characterized steroid-binding properties represent a "bench-mark data set" used extensively for *in silico* studies of protein–ligand interactions and drug design. Recent crystal structure analyses of intact rat CBG and cleaved human CBG have revealed the precise topography of the steroid-binding site, and shown that cortisol-bound CBG displays a typical stressed (S) serpin conformation with the reactive center loop (RCL) fully exposed from the central β-sheet A, while proteolytic cleavage of the RCL results in CBG adopting a relaxed (R) conformation with the cleaved RCL fully inserted within the protein core. These crystal structures have set the stage for mechanistic studies of CBG function which have so far shown that helix D plays a key role in coupling RCL movement and steroid-binding site integrity, and provided evidence for an allosteric mechanism that modulates steroid binding and release from CBG. These studies have also revealed how the irreversible release of steroids occurs after proteolysis and re-orientation of the RCL within the R conformation. This recent insight into the structure and function of CBG reveals how naturally occurring genetic CBG mutations affect steroid binding, and helps understand how proteolysis of CBG enhances the targeted delivery of biologically active steroids to their sites of action.

Contents

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1. Corticosteroid-binding globulin (CBG): a serine proteinase inhibitor (serpin) super-family member with unique biological properties

Corticosteroid-binding globulin (CBG) is a monomeric glycoprotein with a single steroid-binding site that binds as much as 90% of circulating cortisol and corticosterone in humans and rats, respectively (Hammond, 1990). Remarkably, CBG shares little sequence homology with other steroid carriers, and is defined as a clade A serine proteinase inhibitor (serpin) family member that also includes the plasma transport protein for thyroxin, thyroxin-binding globulin (TBG) (Law et al., 2006). This was first realized when it was discovered that the primary structure of CBG shares a remarkable level of sequence identity with α1-antitrypsin (AAT) and α1-antichymotrypsin (ACT), both of which are clade A serpins with well defined serine proteinase inhibitory properties (Hammond et al., 1987). By contrast, CBG and TBG do not appear to act as proteinase inhibitors but rather serve as substrates for specific proteinases like neutrophil elastase (Pemberton et al., 1988; Hammond et al., 1990a), the activity of which is normally controlled by AAT (Hammond et al., 1991).

This close relationship between CBG and AAT/ACT extends to the organization of their genes (Seralini et al., 1990), which are clustered together with 10 other related genes and pseudogenes within a serpin A gene locus of about 370 kb of genomic DNA on human chromosome 14q32.1 (Namciu et al., 2004) that exhibits a high degree of synteny between species. This serpin gene locus can be further subdivided into three sub-clusters of related genes, the most proximal of which spans about 90 kb of genomic DNA that includes the AAT (SERPINA1) gene, an AAT-related pseudogene (SERPINA2), the CBG (SERPINA6) gene, and a protein Z-dependent protease inhibitor (SERPINA10) gene (Namciu et al., 2004). The transcriptional activation of this serpin gene cluster appears to be coordinately regulated by a locus control region upstream of the AAT gene (Zhao et al., 2007) that responds to transcription factors like hepatic nuclear factor (HNF)-1 and HNF-4 involved in mediating important physiological responses, for instance in relation to inflammation (Rollini and Fournier, 1999).

The recognition that CBG and AAT are so closely related, at both the gene and protein levels, reinforces the concept that CBG likely appeared during the evolution of vertebrate species as a result of a gene duplication event (Hammond, 1990). When this occurred during evolution is unclear but it is of interest that mammals, reptiles and birds all appear to have CBG in blood, while claims that fish also have a CBG-like protein in their blood have never been substantiated (Seal and Doe, 1963; Westphal, 1986; Breuner and Orchinik, 2002). The fact that AAT and CBG are targets for the same serine proteinase, neutrophil elastase, and are both classified as "acute phase response proteins" during infectious and inflammatory diseases (Hammond et al., 1991), implies that CBG has evolved to complement the activities of AAT in the control of inflammation. This is a reasonable assumption because the steroid-binding specificity of CBG exhibits a preference for anti-inflammatory steroids, i.e., the glucocorticoids and progesterone (Hammond et al., 1991). Although CBG is produced by hepatocytes in the liver, the CBG gene is also expressed in several other tissues during development, including the kidney and pancreas (Scrocchi et al., 1993a,b). The biological significance of CBG production by these extra-hepatic tissues is not well understood, but it does not appear to contribute to plasma CBG levels and likely serves to control the tissue availability of steroids locally rather than systemically. In this context, the timing of CBG expression in organs like the kidney is of interest because it coincides with periods of active tissue remodeling involving substantial proteolytic activities (Scrocchi et al., 1993a,b).

While AAT interacts with neutrophil elastase to form a covalent one-to-one inhibitory complex after cleavage between residues designated as P1-P1′ of a surface-exposed reactive center loop (RCL) (Johnson and Travis, 1978; Carrell et al., 1982), the RCL region of CBG is simply cleaved by neutrophil elastase without forming an inhibitory complex (Pemberton et al., 1988; Hammond et al., 1990a), and the kinetics of these reactions between neutrophil elastase and AAT or CBG are extremely rapid (Brantly et al., 1988). It should also be noted that in the presence of free radicals, Met358 at P1 in the RCL of human AAT is oxidized and is resistant to neutrophil elastase cleavage at this position (Johnson and Travis, 1978; Johnson and Travis, 1979). Importantly, this prevents AAT from forming an inhibitory complex with this proteinase, and the RCL of oxidized AAT is instead cleaved by elastases at a location corresponding to the P6–P7 residues, and this essentially inactivates AAT as a proteinase inhibitor (Johnson and Travis, 1978, 1979; Carrell et al., 1982; Banda et al., 1987). A methionine is not present within the CBG RCL and its recognition by neutrophil elastase is therefore not influenced by the presence of superoxide radicals. This is considered physiologically important because the plasma concentrations of AAT are much greater that those of CBG, and oxidation of the AAT RCL at sites of inflammation would result in increased exposure of CBG to its possible attack by neutrophil elastase (Hammond, 1990).

It is well established that the cleavage of CBG by neutrophil elastase results in the irreversible destruction of the steroid-binding site, which is accompanied by a marked increase in thermo-stability and a decrease in steroid-binding affinity (Pemberton et al., 1988; Hammond et al., 1990a,b). Since most glucocorticoids in the blood are bound to CBG, this would allow a very substantial increase in the local concentrations of free glucocorticoids at specific sites of action where CBG undergoes proteinase attack. Thus, all the evidence suggests that CBG in the blood serves as a reservoir of anti-inflammatory steroids that can be rapidly released at sites of inflammation in a targeted manner (Hammond, 1990). Although this has been difficult to demonstrate *in vivo*, recent studies of a *cbg* knockout mice have indicated that these animals are much more sensitive to an acute inflammatory challenge than their wild-type counterparts (Petersen et al., 2006). This finding lends credence to earlier observations that some strains of mice, such as BC57BL/6 mice which have relatively low levels of CBG in their blood, are much more sensitive to lethal shock induced by tumor necrosis factor, and that this genetic trait is linked to the *cbg* locus (Libert et al., 1999).

Until recently, our understanding of the biochemical and molecular properties of CBG have been hampered by the lack of a tertiary structure of CBG in complex with a steroid ligand. Early attempts to obtain a CBG crystal structure through the purification of plasma CBG in complex with cortisol failed, most likely because of variations in the degree and composition of N-linked oligosaccharides that decorate the protein isolated from plasma. Expressing proteins in bacteria can circumvent this problem, but attempts to do this with human CBG have not yet met with success. The reason for this is unclear and we initially considered that this might be an impossible task because our early biochemical analyses of human CBG glycosylation-deficient mutants suggested that the utilization of a phylogenetically conserved N-glycosylation site might be important for the correct folding of CBG during synthesis, at least in mammalian cells (Avvakumov et al., 1993; Avvakumov and Hammond, 1994a). However, this may not be correct because rat CBG can be produced in bacteria in a complex with cortisol, and crystallization of this rat CBG:cortisol complex allowed us to define the structure and topography of the steroid-binding site, as well as a plausible mechanism for facilitated steroid binding and release under normal homeostatic conditions or after proteolysis of the RCL (Klieber et al., 2007). Further insight into CBG