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Homology model of human corticosteroid binding globulin: a study of its steroid binding ability and a plausible mechanism of steroid hormone release at the site of inflammation

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Abstract Corticosteroid binding globulin (CBG) and thyroxin binding globulin (TBG) both belong to the same SERPIN superfamily of serine–proteinase inhibitors but in the course of evolution CBG has adapted to its new role as a transport agent of insoluble hormones. CBG binds corticosteroids in plasma, delivering them to sites of inflammation to modify the inflammatory response. CBG is an effective drug carrier for genetic manipulation, and hence there is immense biological interest in the location of the hormone binding site. The crystal structure of human CBG (hCBG) has not been determined, but sequence alignment with other SERPINs suggests that it conforms as a whole to the tertiary structure shared by the superfamily. Human CBG shares 52.15% and 55.50% sequence similarity with α1-antitrypsin and α1-antichymotrypsin, respectively. Multiple sequence alignment among the three sequences shows 73 conserved regions. The molecular structures of α1-antitrypsin and α1-antichymotrypsin, the archetype of the SERPIN superfamily, obtained by X-ray diffraction methods are used to develop a homology model of hCBG. Energy minimization was applied to the model to refine the structure further. The homology model of hCBG contains 371 residues (His13 to Val383). The secondary structure comprises 11 helices, 15 turns and 11 sheets. The putative corticosteroid binding region is found to exist in a pocket between β-sheets S4, S10, S11 and α helix H10. Both cortisol and aldosterone are docked to the elongated hydrophobic ligand binding pocket with the polar residues at the two extremities. A difference accessible surface area (DASA) study revealed that cortisol binds with the native hCBG more tightly than aldosterone. Cleavage at the Val379–Met380 peptide bond causes a deformation of hCBG (also revealed through a DASA study). This deformation could probably trigger the release of the bound hormone.

Keywords Homology model · Transcortin · Hormone release · Proteolytic cleavage

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Introduction

The main plasma-binding protein is an α-globulin called transcortin or corticosteroid binding globulin (CBG). The major site of CBG biosynthesis in adults is clearly the liver, and the human CBG gene promoter contains sequence elements that interact with liver-specific transcription factors. [1] CBG is produced in the liver, and its synthesis is increased by estrogens. Specific binding sites for CBG and its pregnancy-associated variant (pCBG), which has a modified carbohydrate moiety, were found in the plasma membranes of human liver, decidual endometrium and placental syncytiotrophoblast. Both normal CBG and pCBG are involved in the guided transport of steroid hormones to the target cells and transmembrane transfer of hormones and/or hormone signals. [2] Cortisol circulates in plasma in protein-bound and free forms. In human blood, cortisol is transported by CBG, which acts as a substrate for neutrophil elastase. CBG is cleaved specifically by this protease at a precise site close to its carboxy terminus. This induces a conformational change in CBG, disrupts the binding between glucocorticoids and CBG, and thereby promotes a significant local release of glucocorticoids to sites of inflammation. [3, 4, 5] CBG, therefore, plays a crucial role in efficient glucocorticoid action in physiology. The elucidation of the CBG sequence, the knowledge of its gene structure, and the discovery of its chromosomal localization near two other SERPIN genes, concordantly demonstrate that CBG is a SERPIN. This suggests that CBG, α1-proteinase inhibitor and α1-antichymotrypsin evolved relatively recently by gene duplication. [1, 4] CBG and thyroxine- binding globulin both belong to the same SERPIN superfamily of serine–proteinase inhibitors but in the course of evolution

CBG has adapted to its new role as a transport agent of insoluble hormones. [6] CBG binds corticosteroids in plasma, delivering them to sites of inflammation to modify the inflammatory response. CBG is an effective drug carrier for genetic manipulation and hence there is immense biological interest in the location of the hormone binding site. [7]

The crystal structure of human CBG (hCBG) has not yet been determined, but sequence alignment with other SERPINs suggests that it conforms as a whole to the tertiary structure shared by the superfamily. [7] The homology model of hCBG was developed based on the X-ray structures of α1-antitrypsin [8] and α1-antichymotrypsin, [9] which both belong to the SERPIN superfamily. The model globulin protein is then used to study the binding site interactions during complexation with cortisol and aldosterone by molecular modeling. The difference in accessible surface area (DASA) [10] between hCBG and the steroid-bound protein is then calculated for both the cortisol–hCBG and aldosterone–hCBG complexes. The regions of interaction between hCBG and its cognate ligand were revealed from the positive DASA values. Negative DASA values are found to be around the zone of deformation, suggesting unfolding of the buried region due to the ligand binding into the hydrophobic elongated pocket.

Primary structure comparisons of CBG and α1-proteinase inhibitor (A1-PI), suggest that CBG also acts as a substrate for neutrophil elastase. However, unlike A1-PI, CBG does not alter the activity of this enzyme, but is itself cleaved by it at a single location close to its carboxy terminus, causing a reduction of its molecular size by 5 kDa with the concomitant release of more than 80% of CBG-bound cortisol. [3] Here we have proposed a plausible site of cleavage to be the V379–M380 peptide bond.

Materials and methods

Starting conformation and sequence alignment

The refined 3-D structures of α1-antitrypsin (2.7 Å resolution) [8] and α1-antichymotrypsin (2.1 Å resolution), [9] obtained from X-ray diffraction, were taken from the Brookhaven Protein Data Bank (PDB entries 1ATU and 1AS4) as starting materials to develop a homology model of hCBG. The amino acid sequences of these two proteins were extracted from their X-ray crystal structures. The amino acid sequence of hCBG [11, 12, 13, 14] was obtained from SWISSPROT Sequence Data Bank and was compared with the sequences of the crystal structures separately by pairwise sequence alignment using the software GAP [15] of the GCG package. A multiple sequence alignment among hCBG, α1-antitrypsin and α1-antichymotrypsin was done using the PILE UP [16] program of the GCG package. The default parameters of the GCG package with regards to GAP and PILE UP programs are given by a GAP weight of 8 and a length weight of 2. The Blossum matrix was used in sequence alignment and the entire process was fully automatic without any manual intervention.

Coordinate assignment and minimization

The coordinates of hCBG were assigned using the X-ray structures of α1-antitrypsin and α1-antichymotrypsin after aligning the three sequences, as found in the output of the PILE UP program of the GCG package. This coordinate assignment was done by the HOMOLOGY module [17] of the Insight II program package. In this module, the coordinates of the residual parts of hCBG are assigned with the most probable relative disposition of the residues following the backbone of the homologous X-ray structure. The model of hCBG was then put through energy minimization for 7,000 steps of steepest descent using the DISCOVER module of Insight II. The model was further subjected to energy minimization for 1,000 steps of the conjugate technique that led to a refined structure of hCBG with an r.m.s. derivative of less than 0.001. During the process of energy minimization the nonbonded cutoff parameter was 15 and the dielectric constant was 1. The secondary structural elements of hCBG were obtained using the program DSSP, [18] July 1995 version. The DISCOVER simulation package (Biosym Technologies) with the consistent valence force-field [19, 20] was employed for minimization calculations.

Superposition and ligand docking

The putative corticosteroid binding region is found to exist in a pocket between β-sheets S4, S10, S11 and α-helix H10. The molecular structures of both cortisol [21] and aldosterone are docked to this elongated hydrophobic ligand binding pocket. This was done by a ligand-docking program GOLD, [22, 23] where a genetic algorithm was used. Both the hCBG–cortisol and the hCBG–aldosterone complexes were then subjected to energy minimization for 1,000 steps of the conjugate gradient technique by the DISCOVER simulation package.

Solvent accessibility

The values of the accessible surface area for the native protein hCBG and its complexes with the cognate ligands cortisol and aldosterone were calculated using the HOMOLOGY module of Insight II. The differences in accessible surface areas between hCBG and the ligand-bound protein were then calculated for every residue for both the complexes. This DASA study traced the steroid–protein interaction regions as well as the zone of deformation arising as a result of binding of hCBG with the cognate ligands. A DASA study between the native protein hCBG and cortisol-bound truncated hCBG

cleaved at the Val379–Met380 peptide bond also suggested deformation.

Results and discussion

hCBG shares 48.3% sequence identity and 55.5% sequence similarity with α1-antichymotrypsin, a typical inhibitory member of the SERPIN family. The hCBG sequence shows 43.3% identity and 52.2% similarity with the sequence of α1-antitrypsin, the proteotypic SERPIN with a molecular weight of ~53 kDa consists of 394 amino acid residues. [24] Multiple sequence alignment among the three SERPINs (two of which were obtained from X-ray crystal structure analysis) shows the 73 different conserved regions shown in Fig. 1. Energy minimization of the model protein (hCBG) converged to a final r.m.s. deviation 0.0002 from the starting value of 10.172. The superpositions of the backbone of hCBG with those of α1-antitrypsin and α1-antichymotrypsin show minimal deviations. It was noted that the r.m.s. deviations in the aligned position are 6.140 and 9.736, respectively. The superposition of the three SERPINs is shown in Fig. 2. From analogy with other ligand binding proteins of

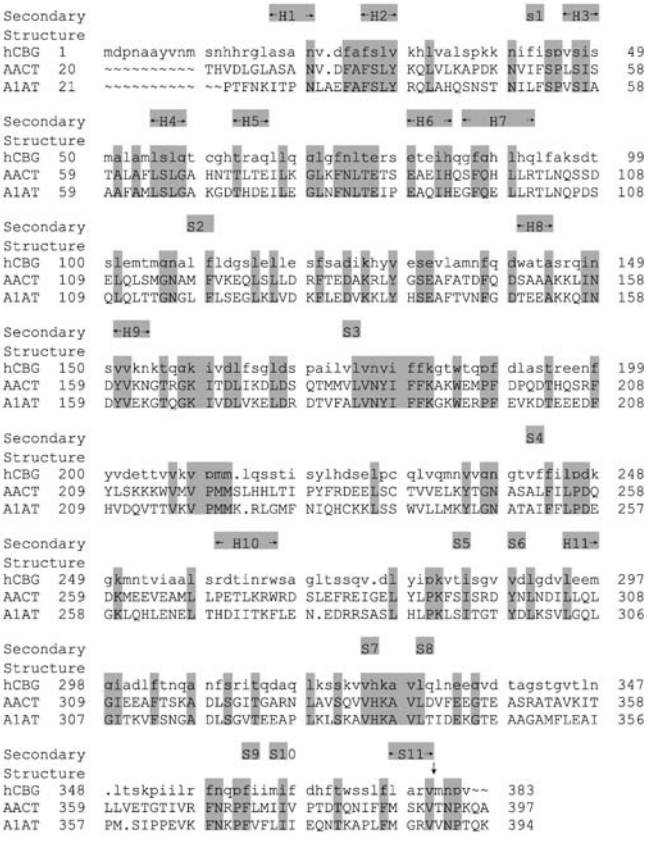

Fig. 1 Multiple sequence alignment among hCBG, AACT and A1AT. *Horizontal shaded* area indicate the secondary structural elements of hCBG. The conserved regions are indicated by *vertical shaded* areas. The *downward arrow* at the C-terminus indicates the site of cleavage

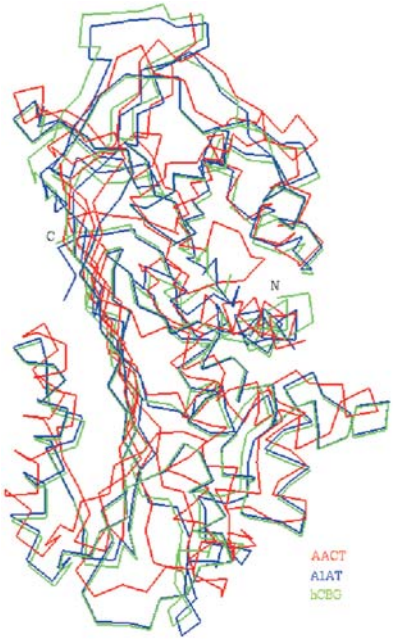

Fig. 2 The superposition of the Cα traces of hCBG, AACT and A1AT. The hCBG is represented in *green*, AACT in *red* and A1AT in *blue*

the same superfamily, it could be expected that for both CBG and TBG the hormone-binding site would lie within a β-barrel. [25] Models of TBG, [26, 27] based on the structure of α1-antitrypsin, support this view, whereas a different binding site in hCBG is observed between the β-sheets S4, S10, S11 and α-helix H10 (Fig. 3a). [7] There are six carbohydrate attachment sites of hCBG at Asn31, Asn96, Asn176, Asn260, Asn330 and Asn369, whereas a1-antitrypsin contains three carbohydrate attachment sites at Asn46, Asn83 and Asn247. Serine protease inhibitors act by complexing with serine proteases, after which they are proteolytically cleaved at their reactive site to form a more stable, inactive species. [6] Here in this serine protease inhibitor hCBG proteolytic cleavage that probably occurs at the Val379–Met380 junction [28] could inactivate it and thereby trigger hormone delivery at the site of inflammation.

52.2% of the residues of hCBG occupy the most favored regions of the Ramachandran Plot and the other residues occupy additional allowed regions as defined in Procheck. [29] No residues of the homology model of hCBG fall in the disallowed region, thereby confirming the reliability of the theoretical model of hCBG. The overall *g* factor as obtained from Procheck is –0.9 Å. The significant number of main-chain torsion angles lying outside the most favored region and a relatively low *g* factor are probably due to similar weaknesses present in the 2.7-Å resolution X-ray structure on the basis of which the homology model of hCBG has been developed.

The postulated model of hCBG consists of 371 residues (His13–Val383), which fold into an elongated hydrophobic ligand-binding pocket with the polar resi-

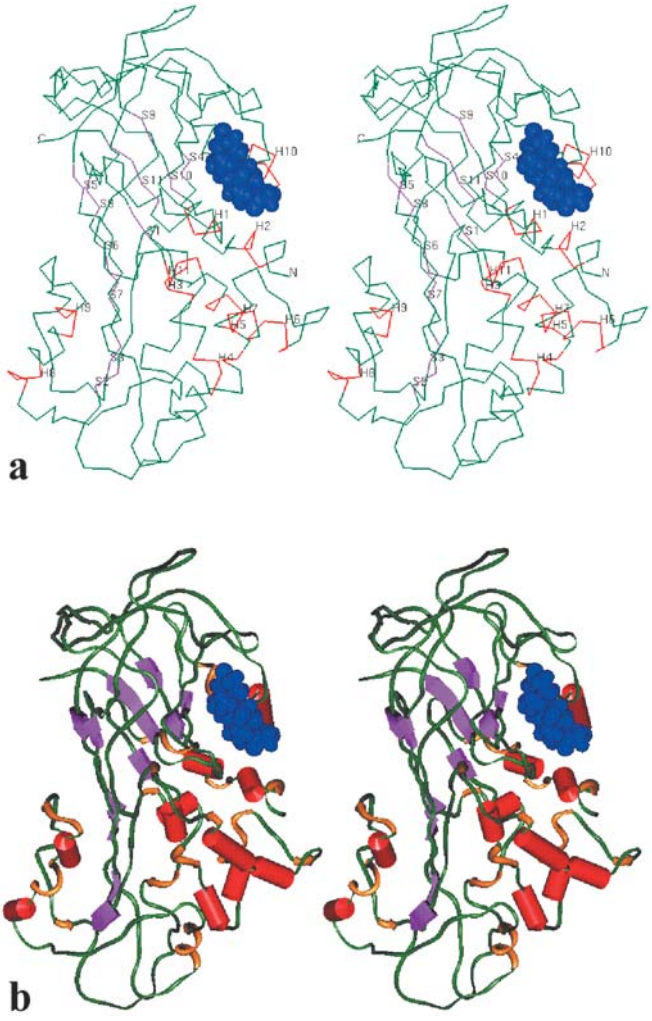

Fig. 3. a Stereoscopic view of the Cα trace of hCBG complexed with cortisol. H and S indicate α-helix and β-sheet respectively. The bound cortisol is shown in a space filling model in *blue*. Helices and sheets are shown in *red* and *magenta* respectively. **b** Stereoscopic view of the ribbon diagram of hCBG complexed with cortisol. The bound cortisol is shown in a space filling model in *blue*. Helices and sheets are shown in *red* and *magenta* respectively. Turns are shown in *yellow*

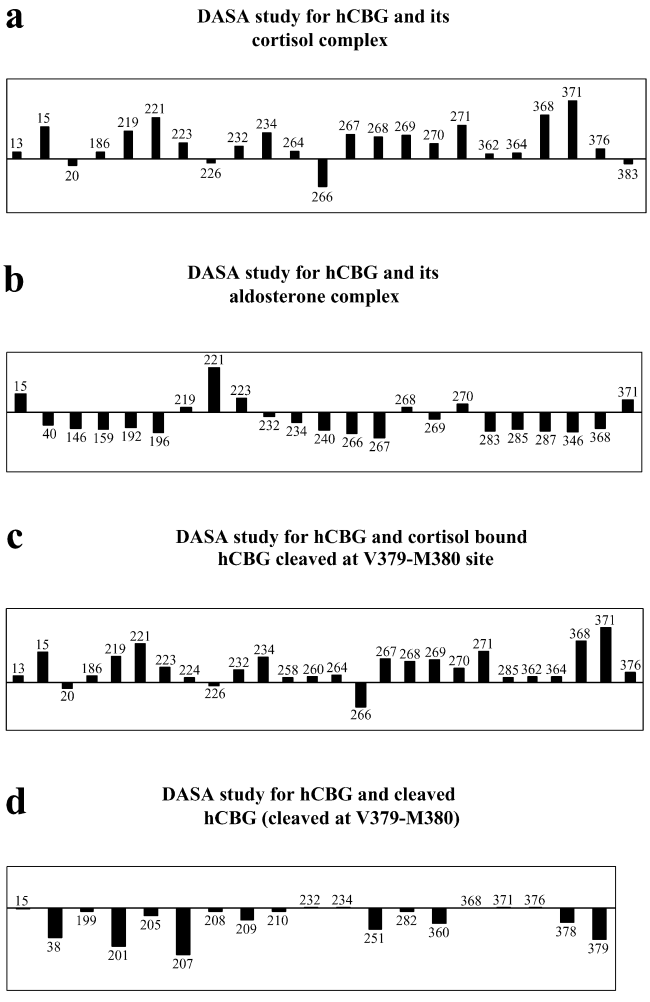

Fig. 4. a DASA study for hCBG and its complex with cortisol. **b** DASA study for hCBG and its complex with aldosterone. **c** DASA study for hCBG and cortisol bound hCBG cleaved at the Val379–Met380 site. **d** DASA study for hCBG and cleaved hCBG (cleaved at the Val379–Met380 site)

dues at the two extremities. This putative binding pocket has a depth of about 26 Å, which agrees with that estimated by electron spin resonance spectroscopy using spin-labeled cortisol analogues. [30] The side chains of Ile263 and Phe369, directed towards the central region of the cavity are mainly hydrophobic in nature, whereas the hydrophilic groups like Ser267, Trp371, Ser219 and Gln232 occupy the two end positions of the ligand-binding pocket. The DASA study between hCBG and its complex with cortisol, a potent glucocorticoid, is shown as a bar graph in Fig. 4a. Figure 4b shows the DASA study between hCBG and its complex with a potent mineralocorticoid aldosterone. The positive DASA values indicate the interaction zone of hCBG with the corticosteroids. A comparative analysis between the DASA studies shown in Fig. 4a and b indicates that the water accessibility of liganded hCBG increases as cortisol is replaced by aldosterone. This is probably due to the lower affinity of aldosterone to hCBG than to cortisol. This result agrees well with that obtained from competitive equilibrium dialysis at pH 7.4, 4 °C, with radiolabeled cortisol. [31] A DASA study shown in Fig. 4c is between unliganded hCBG and cortisol-bound hCBG cleaved at the Val379–Met380 site. The negative DASA values in this figure suggested an increase of water accessibility over a considerable region, owing to cleavage of cortisol bound hCBG at the proposed site. This increase of water accessibility probably arises as a result of deformation of hCBG due to cleavage. A DASA study (Fig. 4d) between hCBG and cleaved hCBG (both unliganded) shows a similar increase in water accessibility, suggesting a similar deformation. The cleavage at the Val379–Met380 site near the carboxy terminus (by neutrophil elastase) deforms the hCBG to trigger the hormone release at the site of inflammation. After repeated

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                                        232    238
    hCBG  215  ss..tisylh dselpcqlvq mnyvgngtvf filpdkgkmn tviaalsrdt  262
mouseCBG  208  sg..nisyfr dsaipcqmvq mnyvgngttf iilpdqgqmd tvvaalnrdt  255
  ratCBG  207  sg..sigyfr dsvfpcqliq mdyvgngtaf filpdqgqmd tviaalsrdt  254
  rabCBG  215  ss..tvkylh dpvlpcrlvq ldyvgngtaf filpdkgkvd tviaalsrdt  262
  pigCBG  214  sr..amkyln dsllpcqlvq leytgnetaf filpvkgemd tviaglsrdt  261

          263 266
    hCBG  263  inrwsaglts sqv.dlyipk vtisgvydlg dvleemgiad lftnqanfsr  311
mouseCBG  256  idrwgklmip rqm.nlyipk fsmsdtydlq dvladvgikd lftnqsdfad  304
  ratCBG  255  idrwgklmtp rqv.nlyipk fsmsdtydlk dvledlnikd lltnqsdfsg  303
  rabCBG  263  iqrwskslty rlv.hlyipk asisgayelr galaamgiad lftnqanfss  311
  pigCBG  262  iqrwskslip sqv.dlyvpk vsisgaydlg silgdmgivd llshpthfsg  310


    hCBG  312  itqdaqlkss kvvhkavlql neegvdtags t...gvtlnl tskpii.lrf  357
mouseCBG  305  ttkdtpltlt .vlhkamlql de.gnvlpaa t..ngppvhl psesft.lky  349
  ratCBG  304  ntkdvpltlt .mvhkamlql de.gnvlpns t..ngaplhl rsepld.ikf  348
  rabCBG  312  isqegplkvs kvlhkavlql dehggvevaa t...ggplql vseplt.lnf  357
  pigCBG  311  itqnalpkms kvvhkavlqf dekgmeaaap t.trgrslha apkpvt.vhf  358

                       369 371       ↓
    hCBG  358  nqpfiimifd hftwsslfla rvmnpv~~  383
mouseCBG  350  nrpfiflafd kvtwsslmms qvmnpa~~  375
  ratCBG  349  nkpfilllfd kftwsslmms qvvnpa~~  374
  rabCBG  358  nrpflilifd dftwsslflg kvvipa~~  383
  pigCBG  359  nrpfivmvfd hftwsslflg kivnlt~~  384
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Fig. 5 Multiple sequence alignment among hCBG, mouse CBG, rat CBG, rabbit CBG and pig CBG. The *vertical shaded* areas indicate the conserved residues having important role in ligand binding and the numbers above indicate the corresponding sequence positions. The *downward arrow* at the C-terminus shows the site of cleavage

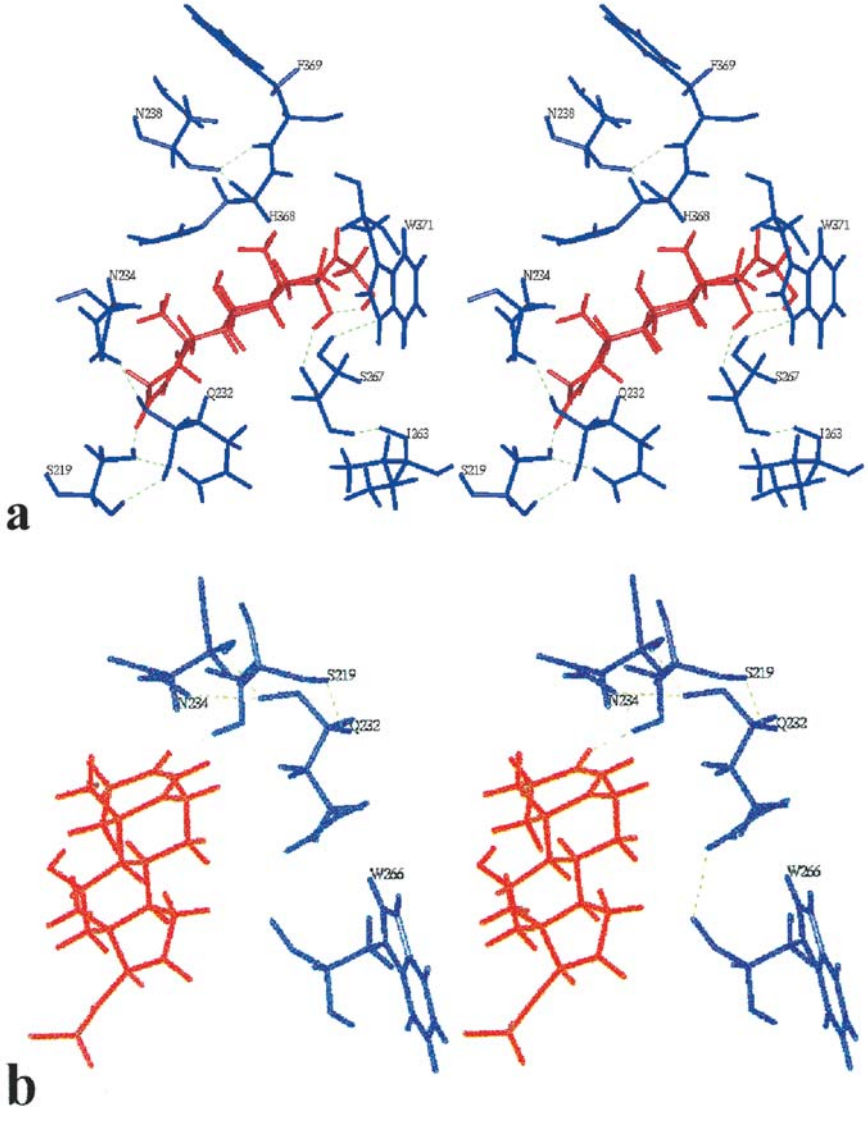

Fig. 6. a Stereoscopic view of the binding sites of cortisol complexed with hCBG in a stick diagram. Here the steroid is shown in *red* and the binding site residues in *blue*. **b** Stereoscopic view of the binding sites of aldosterone complexed with hCBG in a stick diagram. Here the steroid is shown in *red* and the binding site residues in *blue*

Table 1 Hydrogen bonding parameters for cortisol and aldosterone in complex with hCBG

Complex	Donor-H	Acceptor	Distance (Å) H...A	Angle (°) D–H...A
Cortisol bound hCBG	Gln 232:HN	Ser 219:O(keto)	2.08	129.07
	Ser 219:HG	Cortisol:O3(keto)	1.74	163.63
	Asn 234:HN	Gln 232:O(keto)	2.24	121.10
	Asn 234:HD22	Gln 232:O(keto)	2.44	146.39
	Phe 369:HN	Asn 238:O(keto)	1.97	145.54
	Ser 267:HN	Ile 263: O(keto)	1.86	150.45
	Cortisol:O17-H	Ser 267: O(keto)	1.89	146.30
	Ser 267:HG	Trp 371:NE1	2.25	149.46
Aldosterone bound hCBG	Gln 232:HN	Ser 219:O(keto)	2.11	126.87
	Ser 219:HG	Aldosterone:O3(keto)	1.70	166.45
	Asn 234:HN	Gln 232:O(keto)	2.23	121.70
	Asn 234:HD22	Gln 232:O(keto)	2.42	144.66
	Gln 232:HE22	Trp 266:O(keto)	2.36	125.46

refinement by energy minimization of the homology model of cortisol-bound hCBG, it was observed that the cortisol moiety instead of residing deep in the core was more like a flap on the hydrophobic pocket. This probably accounts for its easy dislodgment when deformation takes place. Fig. 3a shows the C_α trace of hCBG, where H and S indicate α-helices and β-sheets, respectively. Bound cortisol is shown in a space-filling model. Figure 3b shows the ribbon diagram of hCBG where the bound cortisol is also shown in a space filling model.

The polar neutral residues Ser219 and Ser267 of hCBG are directly involved in binding with cortisol through hydrogen bonding. The C-3-ketone group of the A-ring of cortisol forms a hydrogen bond with the hydroxyl group in the side chain of Ser219 at site I of the ligand-binding pocket. Ser219 is held in the optimum position by Gln232, to which it is hydrogen bonded. The NH group of Gln232 donates hydrogen to the keto oxygen of Ser219. Gln232 is in turn hydrogen bonded to Asn234. Here, the amido NH_2 group of Asn234 serves as a donor to the keto oxygen of Gln232. It appears that this hydrogen-bonding network contributes to the conformational stability of this region. It is noteworthy that the polar neutral residue Gln232 is conserved at the corresponding sequence position in CBGs of different species (Fig. 5).

The hydroxyl group at C17 of cortisol is hydrogen bonded to the keto oxygen of Ser267 at site II of the ligand-binding pocket. The hydroxyl group in the side chain of Ser267 residue is in turn hydrogen bonded to the nitrogen in the heterocyclic ring of Trp371, while its amido group is hydrogen bonded to the keto oxygen of Ile263. Once again, this hydrogen-bonding network play a crucial role in ligand binding. Trp371 and Ile263 plays a vital role in ideally setting up Ser267 so that the latter can bind to the cortisol. Trp371 and Ile263 are conserved in different species of CBG (Fig. 5). The hydrophobic residue Phe369 and the polar neutral residue Asn238 are found in the ligand-binding pocket and could play a secondary role as both the residues are conserved at the corresponding sequence positions in different species of CBG (Fig. 5).

In the hCBG-aldosterone complex, Ser219 is directly involved in forming a hydrogen bond with the potent mineralocorticoid aldosterone. Here Ser219, Gln232 and Asn234 play a similar role in protein–ligand interaction at site I of the ligand-binding pocket. Additionally, Trp266 also contributes to the stability of this region by forming a hydrogen bond with Gln232. However, no hydrogen-bonding interactions were observed at site II of the ligand-binding pocket, suggesting a relatively loose binding of aldosterone with hCBG compared to that observed in the case of cortisol and confirming the observation made from the DASA study (Fig. 4b). The role of Asn238, Trp266, Trp371 in ligand binding was also observed by earlier workers. [14, 32, 33, 34] Figure 6a and b show a stereoscopic view of the binding-site interactions of cortisol and aldosterone with hCBG. The hydrogen-bonding parameters associated with cortisol and aldosterone in complexation with hCBG are given in Table 1.

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