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Homology modelling of the ligand-binding domain of glucocorticoid receptor: binding site interactions with cortisol and corticosterone

Raja Dey[1], P.Roychowdhury[1,2] and C.Mukherjee[3]

[1]Department of Physics and [3]Department of Chemistry, University of Calcutta, 92 A.P.C. Road, Calcutta-700009, India

[2]To whom correspondence should be addressed.
Email: prcxray@cubmb.ernet.in and draja63@yahoo.co.in

Glucocorticoids are involved in the growth, development and homeostasis of a number of tissues. The physiological effects of this class of lipophilic steroids are mediated by ligand-inducible nuclear transcription factor, the glucocorticoid receptor/mineralocorticoid receptor, a member of the steroid/nuclear receptor superfamily. The glucocorticoid receptor interacts specifically with glucocorticoids, whereas the mineralocorticoid receptor interacts with both glucocorticoids and mineralocorticoids. The molecular structure of progesterone complexed to its receptor obtained from X-ray crystal structure analysis is used to build up a homology model of mouse glucocorticoid receptor ligand-binding domain (mGR LBD). The secondary structure of mGR LBD contains 11 helices, nine turns and four sheets. The mGR LBD contains a long helix, H9, with 30 residues, and exhibits slight deformation when the receptor protein binds with its cognate ligands. The mGR LBD has a 12-residue C-terminal extension (residues 772–783) that is essential for hormone binding. This extension is tightly fixed in position by an antiparallel β-sheet interaction between amino acids 680–682 (S3) and 775–777 (S4). The three-dimensional model reveals two polar sites located at the extremities of the elongated hydrophobic ligand-binding pocket. Cortisol and corticosterone are docked to this ligand-binding pocket. The difference accessible surface area study revealed the steroid-binding region of mGR LBD.

Keywords: glucocorticoid receptor/homology modelling/ligand-binding domain/steroid receptor complex/transactivation

Introduction

The ability of nuclear receptor to activate specific gene transcription requires the binding of cognate ligands to their ligand-binding domains (LBDs) (Wurtz *et al.*, 1996). Glucocorticoid receptor is a member of a family of steroid/nuclear receptors. The steroid receptors, members of a superfamily of eukaryotic transcription factors, regulate gene expression in response to binding small, hydrophobic ligands (Evans, 1988; Tsai and O'Malley, 1994; Ribeiro *et al.*, 1995). Their structure can be divided into three functionally separable domains. The central domain, known as the DNA-binding domain (DBD), binds to specific hormone response elements in the DNA (Evans, 1988; Glass, 1994; Tsai and O'Malley, 1994). The N-terminal domain (NTD) containing activation function 1 (AF1) mediates transactivation (Lind *et al.*, 1999). The third C-terminal domain, known as the ligand-binding domain (Evans, 1988; Laudet *et al.*, 1992), binds the steroids (Carlstedt-Duke *et al.*, 1987) and it contains activation function 2 (AF2) and so it has also a mediatary effect on transactivation. Steroid receptors interact with coactivators during the recruitment of active transcription initiation complexes required for hormone-regulated gene transcription (Torchia *et al.*, 1998; He *et al.*, 1999). Recent studies have focused on a family of p160 coactivators, that interact with the AF2 region of the LBD of steroid receptors, and include steroid receptor coactivator 1 (SRC1) (Onate *et al.*, 1995) and human transcriptional intermediary factor 2 (TIF2) (Voegel *et al.*, 1998). The NTD physically interacts with nuclear receptor coactivators and with the LBD and this interaction, like the functional interaction between the LBD and p160 coactivators, relies on the AF2 core of the activating domain (AD) (Alen *et al.*, 1999). Binding of the steroid actively modulates the structure of the receptor into a DNA-binding transcriptionally active complex (Wagner *et al.*, 1995; Parker and White, 1996; Brzozowski *et al.*, 1997). The steroid hormone is an integral part of the transcriptionally active receptor–ligand complex and is almost completely buried within the fold of the LBD.

The DBDs and LBDs for almost all the members of the steroid receptor superfamily are relatively well conserved, whereas the NTDs are less conserved (Hollenberg *et al.*, 1985; Arriza *et al.*, 1987). The receptors have overlapping steroid-binding specificity as they bind to the same hormone-responsive elements in target genes (Arriza *et al.*, 1987; Pearce and Yamamoto, 1993; Rupprecht *et al.*, 1993b). Mineralocorticoid receptor binds mineralocorticoids and most glucocorticoids with high affinity, whereas glucocorticoid receptor binds only glucocorticoids with high affinity (Arriza *et al.*, 1987, 1998; Rupprecht *et al.*, 1993a,b). There is a functional similarity in both these classes of steroids, but glucocorticoids specifically regulate the development of specific tissues, glucose metabolism and the immune response (Miller and Tyrrell, 1995).

A remarkable feature of the glucocorticoids is that they exert pronounced effects on a variety of tissues. In certain sites they stimulate the synthesis of macromolecular components, including nucleic acids and proteins. Sometimes the action of glucocorticoids is catabolic, resulting ultimately in cellular destruction (King, 1974). This diversity of action is reflected in the contemporary search for specific glucocorticoid-binding proteins in a wide range of experimental systems. Binding to the specific hormone receptors is acutely dependent on the

Table I. Similarities and identities of the sequences of mGR with those of hPR LBD, hAR LBD and hER LBD obtained by GAP software of the GCG package

Pairwise sequence alignment between	Similarities	Identities
mGR and hPR LBD	66.275	54.902
mGR and hAR LBD	37.476	29.190
mGR and hER LBD	42.629	36.255

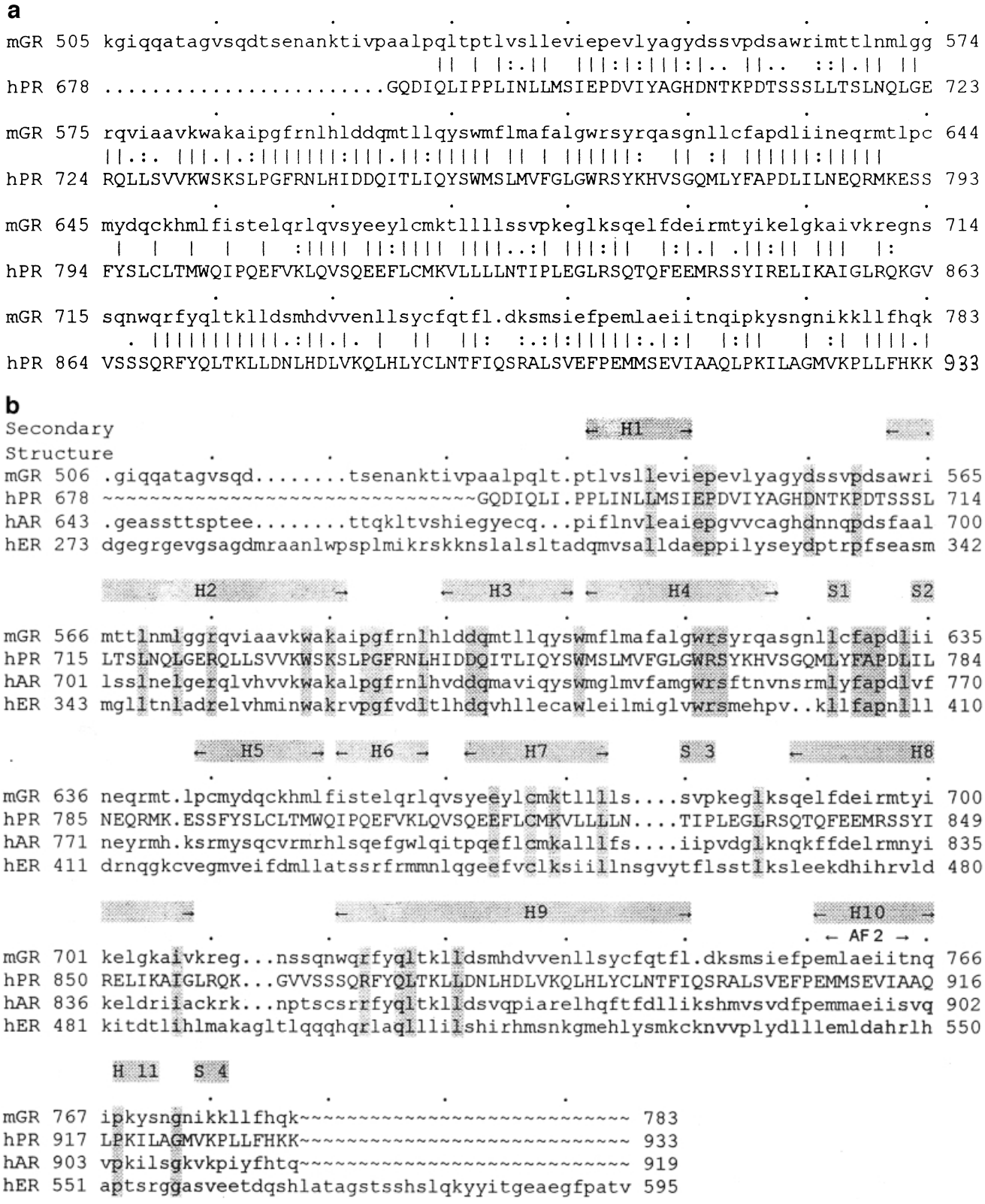

Fig. 1. (**a**) Pairwise sequence alignment between mGR and hPR LBD. The solid vertical lines between the sequences indicate the identities. Double dots and single dots indicate high and low similarities between the corresponding amino acids, respectively. (**b**) Multiple sequence alignment among mGR, hPR LBD, hAR LBD and hER LBD. Horizontal shaded areas indicate the secondary structural elements of mGR LBD. The conserved regions are indicated by vertical shaded areas.

presence of the 3-one A-ring and/or 11β-hydroxyl group, which is the characteristic structural feature of all biologically active glucocorticoids. The 3-one A-ring of many progestins and corticoids is the area of greatest conformational flexibility in these molecules. This conformational flexibility exhibited by these naturally occurring hormones facilitates their highly

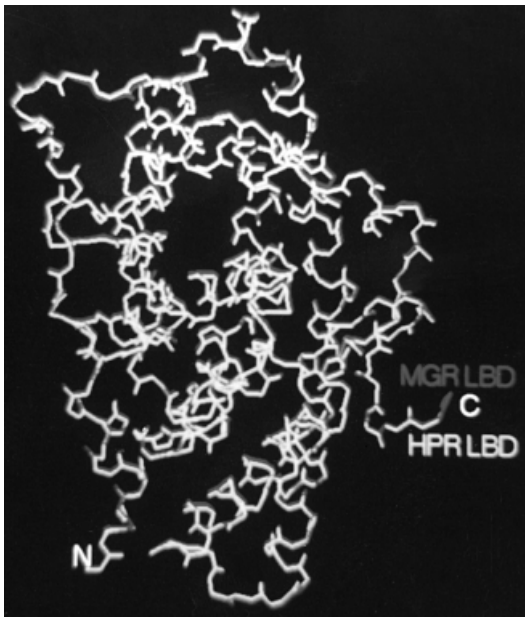

Fig. 2. Superposition of the backbone of mGR LBD on hPR LBD.

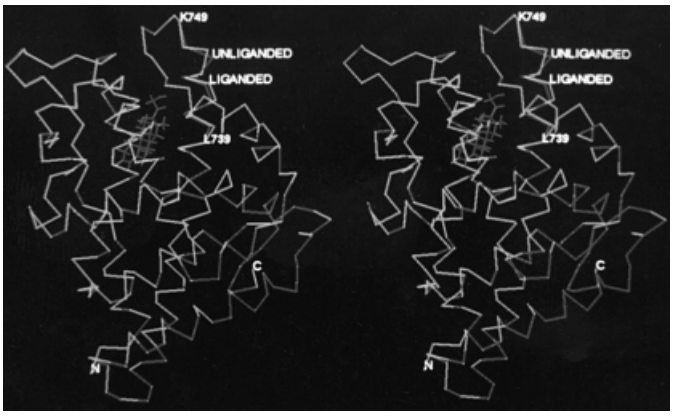

Fig. 3. Stereoscopic view of superposition of the trace of mGR LBD–cortisol complex on the uncomplexed mGR LBD.

specific interaction with a variety of proteins including synthesizing enzymes, transport proteins and target proteins (Duax and Norton, 1975).

The theoretical model of mGR LBD was developed templating the X-ray structure of progesterone receptor (Williams and Siglar, 1998). The model receptor protein is then used to study the binding site interactions during complexation with cortisol and corticosterone by molecular modelling. The difference in accessible surface area (DASA) (Lee and Richards, 1971) between mGR LBD and the ligand-bound protein is then calculated for both the steroid–receptor complexes. The interaction zone of mGR LBD with the steroid was revealed from this study. Binding of steroids to mGR LBD does not necessitate any structural change to the hydrophobic ligand-binding pocket. However, the modelling study did indicate a slight conformational change in the secondary structure on complexation of mGR LBD with both cortisol and corticosterone. This minor conformational change was found in the C-terminal site of the long helix H9 without any gross alteration of the ligand-binding pocket itself. This suggests that ligand binding may trigger a conformational modification which could account for the effect of ligand binding on transactivation by AF-2

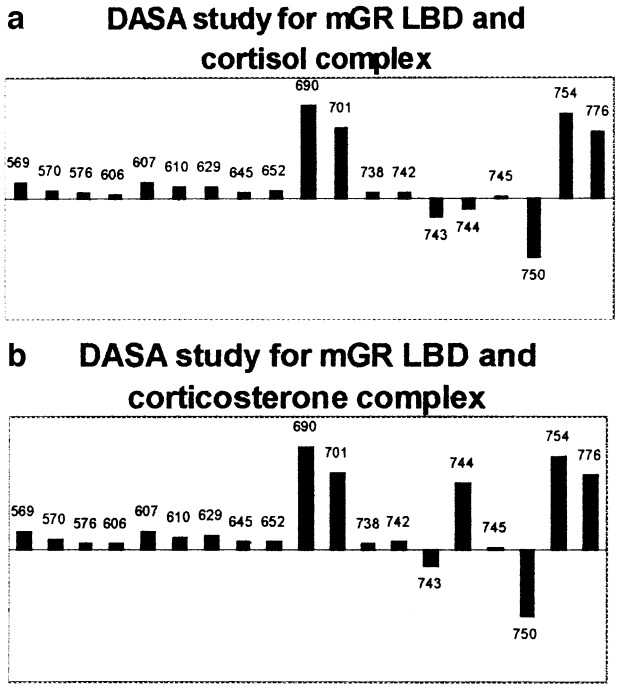

Fig. 4. DASA studies of mGR LBD and its complex with (**a**)cortisol and (**b**) corticosterone.

(Bourguet *et al.*, 1995). Perhaps the ability of corticosteroids to stimulate the transactivation function of mGR depends on the stability of the steroid–receptor complexes (Hellal-Levy *et al.*, 1999).

Materials and methods

Starting conformation and sequence alignment

The refined crystal structures of progesterone-bound LBD of the human progesterone receptor (hPR) (Williams and Siglar, 1998), LBD of estrogen receptor (ER) in complex with the endrogenous estrogen (Brzozowski *et al.*, 1997) and the LBD of human androgen receptor (hAR) in complex with metribolone (R1881) (Matias *et al.*, 2000) were taken from the Brookhaven Protein Data Bank (PDB entries 1A28, 1ERRand 1e3g, respectively) as starting materials. The amino acid sequences of three LBDs were extracted from these three crystal structures. The amino acid sequence of house mouse glucocorticoid receptor (mGR) (Danielsen *et al.*, 1986; Nohno *et al.*, 1989) 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 (Needleman and Wunsch, 1970) of the GCG package. The best similarity and identity of mGR with hPR LBD encouraged us to select the crystal structure of progesterone-bound LBD of hPR to develop a theoretical model of mGR LBD. A multiple sequence alignment among mGR, hPR, hAR and hER was done using the PILE UP (Feng and Doolittle, 1987) program of the GCG package.

Coordinate assignment and minimization

The coordinates of mGR LBD were assigned by templating the X-ray structure of hPR LBD after aligning the two sequences as was observed in the GCG output. This coordinate assignment was done using the HOMOLOGY module (Biosym Technologies) of the InsightII program package. The model of mGR LBD was then put through energy minimization

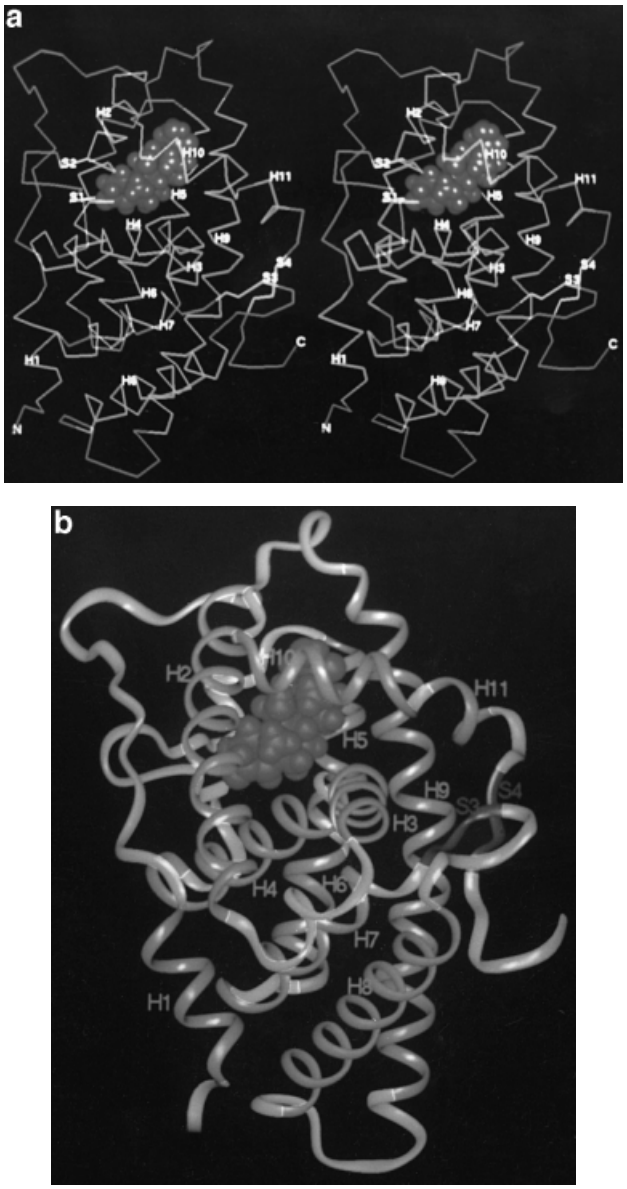

Fig. 5. (**a**) Stereoscopic view of the Cα trace of mGR LBD complexed with cortisol. H and S indicate α-helix and β-sheet, respectively. The bound cortisol is shown in a space filling model. (**b**) Ribbon diagram of mGR LBD complexed to cortisol. The bound cortisol is shown in a space filling model.

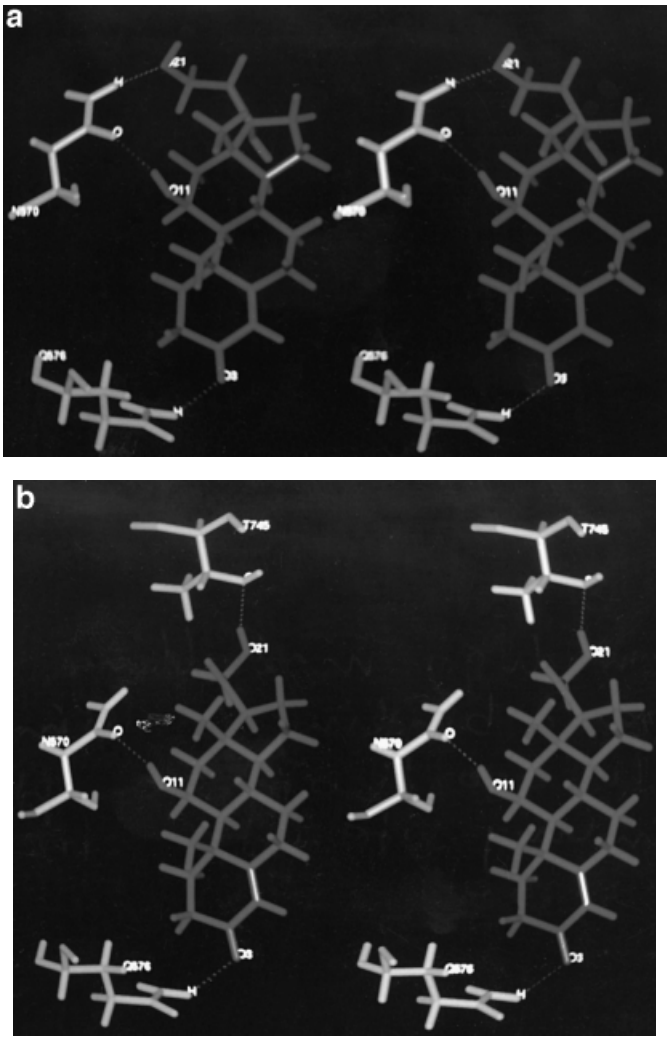

Fig. 6. (**a**) Stereoscopic view of the binding sites of cortisol complexed with mGR LBD in a stick diagram. (**b**) Stereoscopic view of the binding sites of corticosterone complexed with mGR LBD in a stick diagram.

for 10 000 steps of the steepest descent method using the DISCOVER module of InsightII. The model was further subjected to energy minimization for 500 steps of the conjugate gradient technique that led to a refined structure of mGR LBD with an r.m.s. deviation of <0.001. The secondary structure prediction of mGR LBD was performed using the program DSSP (Kabsch and Sander, 1983), July 1995 version. The DISCOVER simulation package (Biosym Technologies) with the consistent valence force-field (Hagler *et al.*, 1985; Dauber-Osguthorpe *et al.*, 1988) was employed for minimization calculations.

Superposition and ligand docking

Using the Biopolymer module (Biosym Technologies) of InsightII, the refined model structure of mGR LBD was superposed on the crystal structure of progesterone-bound hPR LBD. The molecular structure of cortisol (Roberts *et al.*, 1973) was then superposed on progesterone such that their steroid nuclei were practically coincident. The allocated cortisol could then be easily associated with the ligand-binding domain of the superposed glucocorticoid receptor. The hydroxyl group at C-17 of the cortisol in the mGR LBD–cortisol complex thus prepared was deleted to mimic an mGR LBD–corticosterone complex. These two complexes were then subjected to energy minimization for 1000 steps of the conjugate gradient technique with the DISCOVER simulation package.

Solvent accessibility

The values of the accessible surface area for both the native protein (mGR LBD) and ligand-bound proteins were calculated using the HOMOLOGY module of InsightII. The differences in accessible surface areas between mGR LBD and ligand-bound protein were calculated for every residue. This DASA study traced the steroid protein interaction regions for the complexes of mGR LBD with both cortisol and corticosterone.

Results and discussion

Table I shows the sequence identities and similarities obtained from pairwise sequence alignment of mGR with hPR LBD, hAR LBD and hER LBD. hPR LBD shows the best pairwise alignment [Figure 1(a)]. The multiple sequence alignment

Table II. Hydrogen-bonding parameters for cortisol and corticosterone in complex with mGR LBD

Complex	Donor-H	Acceptor	H···A distance (Å)	D–H···A angle (°)
mGR LBD–cortisol	Steroid: O11–H	Asn570: O (keto)	2.09	167.12
	Asn570: N–H	Steroid: O-21	1.96	163.88
	Gln576: N–H	Steroid: O-3	2.30	137.66
mGR LBD–corticosterone	Steroid: O-11–H	Asn570: O (keto)	1.95	162.01
	Gln576: N–H	Steroid: O-3	2.41	135.87
	Steroid: O-21–H	Thr745: O (hydroxy)	1.92	156.19

Table III. Binding energies of cortisol and corticosterone in their complexes with mGR LBD

Final total energy for the uncomplexed receptor (E_r)		Final total energy for the steroid (E_s)		Final total energy for the complexed receptor (E_c)		Binding energy of steroid to receptor (E_b)[a]	
Receptor	E_r (kcal)	Steroid	E_s (kcal)	Complex	E_c (kcal)	Steroid	E_b (kcal)
mGR LBD	–47299.07	Cortisol	85.23	mGR LBD–cortisol	–46775.01	Cortisol	–438.83
mGR LBD	–47299.07	Corticosterone	57.89	mGR LBD–corticosterone	–46804.46	Corticosterone	–436.72

[a] $E_b = (E_r + E_s) - E_c$.

Table IV. Final energies associated with the uncomplexed receptors mGR LBD and hPR LBD

Uncomplexed receptor	Bond energy (kcal)	Theta energy (kcal)	Phi energy (kcal)	Out-of-plane energy (kcal)	Non-bond energy (kcal)	Coulomb energy (kcal)	Total energy (kcal)
mGR LBD	636.76	1393.30	196.40	13.40	119.92	–49658.84	–47299.07
hPR LBD	387.47	994.97	182.73	3.27	–18.47	–49569.36	–48019.39

Table V. Final energies associated with the receptors mGR LBD and hPR LBD complexed to pregnen steroids

Complex of	Bond energy (kcal)	Theta energy (kcal)	Phi energy (kcal)	Out-of-plane energy (kcal)	Non-bond energy (kcal)	Coulomb energy (kcal)	Total energy (kcal)
mGR LBD–cortisol	648.67	1383.35	226.90	13.14	176.11	–49223.18	–46775.01
mGR LBD–corticosterone	647.36	1383.93	225.07	13.18	177.23	–49252.04	–46804.46
hPR LBD–progesterone	753.36	1330.55	194.15	10.98	–158.43	–48226.07	–46095.45

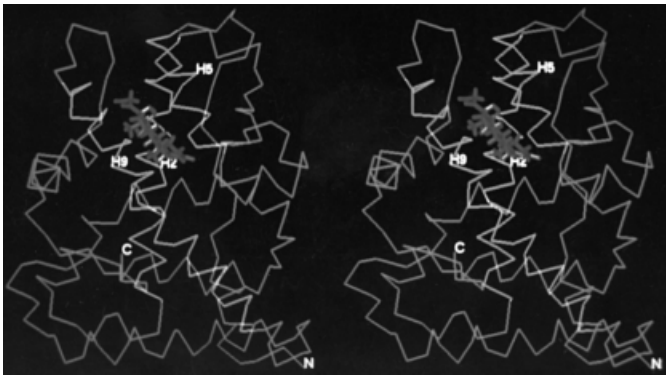

Fig. 7. Stereoscopic view of the disposition of the helices H2, H5 and H9 around cortisol complexed with mGR LBD. Here the receptor is shown as a trace and the cortisol as a stick diagram.

among these four receptors (three of which were obtained from X-ray analysis) shows 28 totally conserved regions represented in Figure 1(b). The r.m.s. deviation dropped from 2.8378 (initial value) to 0.0003 (final value) after energy minimization of the model receptor. The superposition (Figure 2) of the backbone of mGR LBD with that of hPR LBD shows minimal deviation. It was noted that the r.m.s. deviation in the aligned position is 1.326.

The majority of the residues of mGR LBD occupy the most favored regions of the Ramachandran plot and the other residues occupy additional allowed regions as defined in Procheck (Laskowski *et al.*, 1993). No residues of the model protein fall in the disallowed region, thereby confirming the reliability of the theoretical model of mGR LBD.

The postulated model of mGR LBD consists of 250 residues (Leu534–Lys783), which folds into a hydrophobic ligand-binding pocket. The secondary structure of this model protein contains 11 helices, nine turns and four sheets. The pairs of helices H3, H4; H5, H6; and H10, H11 are almost contiguous pairwise. mGR LBD contains a relatively longer helix H9 (it contains 30 residues, whereas the PR contains 15, the RAR contains eight, the TR contains six and the ER contains nine). A shortening of the length of the helix H9 is observed in both the complexes of mGR LBD with cortisol and corticosterone. On complexation the helix H9 (718–747) of the native protein (mGR LBD) becomes deformed and is shortened by one residue to become helix H9 (718–746). This deformation at the C-terminal end of helix H9 is shown in Figure 3, where the trace of mGR LBD–cortisol complex is superposed on the

native protein. This superposition (Figure 3) shows a perfect coincidence everywhere except from Ser740 to Asp748. The r.m.s. deviation in the aligned position is 0.147. The DASA study between mGR LBD and its complex with cortisol is represented by the bar graph in Figure 4(a). Figure 4(b) represents the DASA study between mGR LBD and its corticosterone complex. The positive DASA values reveal the interaction zone of the receptor with its cognate ligands. The negative DASA values are found to be around the region of deformation arises from complexation. Figure 5(a) represents the Cα trace of mGR LBD, where H and S indicate α-helices and β-sheets, respectively. Bound cortisol is shown in a space filling model. Figure 5(b) represents the ribbon diagram of mGR LBD where the bound cortisol is shown in the space filling model.

The polar neutral residues Asn570 and Gln576 of mGR LBD 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 amido NH_2 group of Gln576 of helix H2 at site I of the ligand-binding pocket. The required position and orientation of Gln576 are maintained by the supporting role of Arg617 and Phe629, underscored by their conservation at the corresponding sequence position in all steroid receptors. The amido NH_2 group of the polar residue Asn570 of helix H2 serves as a donor to O-21 of cortisol and the keto-oxygen of the same residue serves as an acceptor from the hydroxyl group at C-11 of cortisol. The residue Asn570 thereby held stably by forming two hydrogen bonds with the steroid at site II of the ligand-binding pocket. The remaining residues in the binding cavity participate in a number of hydrophobic interactions with the ligand. The mineralocorticoid compound aldosterone bearing an identical 17β-substituent as in cortisol shows similar binding interactions with MR in site II of the ligand-binding pocket (Fagart *et al.*, 1998). The residues of mGR LBD that are directly involved in complexation with corticosterone through a hydrogen-bonding network are Asn570, Gln576 and Thr745. Here the A-ring of corticosterone is directed towards site I of the ligand-binding pocket where the polar residue Gln576 of helix H2 forms a hydrogen bond with the keto-oxygen at C-3 of the steroid. The hydroxyl groups at C-11 and C-21 of corticosterone serve as donors to the keto-oxygen of Asn-570 and the hydroxyl group of Thr-745, respectively, and thereby forms two hydrogen bonds at site II of the ligand-binding pocket. The binding study of mGR LBD with its cognate ligands shows that the polar neutral residues Asn570 and Gln576 play a key role in complexation.

The hydrogen-bonding parameters associated with cortisol and corticosterone in complexation with mGR LBD are given in Table II. Table III presents the binding energies of cortisol and corticosterone in their complexes with mGR LBD. Figure 6(a) and (b) show a stereoscopic view of the binding site interactions of cortisol and corticosterone, respectively, with mGR LBD in a stick diagram. Final energies before complexation of mGR LBD and hPR LBD (X-ray structure) with steroids are given in Table IV. Table V represents the final energies of the receptors complexed to steroids. Tables IV and V clearly reveal a high degree of agreement between the actual crystal structure and model structure, thereby confirming the reliability of the modelled steroid–receptor complex as well as the model of the uncomplexed mGR LBD.

The overall architechture of this modeled mGR LBD is similar to that seen in the crystal structures of the LBDs of other nuclear receptors such as ER, TR, PR, RXR, AR and RAR (Bourguet *et al.*, 1995; Renaud *et al.*, 1995; Wagner *et al.*, 1995; Brzozowski *et al.*, 1997; Williams and Siglar, 1998; Matias *et al.*, 2000). The structures of all these LBDs are folded into a three-layered antiparallel β-helical sandwich. Like most 3-keto steroid receptors, the mGR LBD has a 12-residue C-terminal extension (772–783), that is essential for hormone binding in GR, PR and AR (Jenster *et al.*, 1991; Xu *et al.*, 1996; Zhang *et al.*, 1996). However, ER is found to be an exception (Lanz and Rusconi, 1996). This extension is tightly fixed in position by an antiparallel β-sheet interaction between amino acids 680–682 and 775–777. The ligand is surrounded by three helices, H2, H5 and H9. The helices H5 and H9 are nearly parallel to the steroid nuclear plane and disposed at the α and β orientational side of the steroid nucleus, respectively. However, the helix H2 is disposed at an angle with the steroid nucleus. The disposition of these three helices around cortisol in complex with mGR LBD is shown in Figure 7. The residues (Met758–Thr764) of mGR LBD corresponds to the AF2-AD core of the X-ray structures of PR LBD as was found in the multiple sequence alignment (Figure 1). Hence the seven residues (Met758–Thr764) may be treated as the AF2-AD core of mGR LBD that belongs to helix H10. This probable AF2-AD core of mGR LBD could affect the conformation at the C-terminal end of the helix H9 in the presence of bound agonist.

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