The Crystal Structure of Guinea Pig 11β-Hydroxysteroid Dehydrogenase Type 1 Provides a Model for Enzyme-Lipid Bilayer Interactions*

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The metabolic reduction of 11-keto groups in glucocorticoid steroids such as cortisone leads to the nuclear receptor ligand cortisol. This conversion is an example of pre-receptor regulation and constitutes a novel pharmacological target for the treatment of metabolic disorders such as insulin resistance and possibly other derangements observed in the metabolic syndrome, such as hyperlipidemia, hypertension, and lowered insulin secretion. This reaction is carried out by the NADPH-dependent type 1 11β-hydroxysteroid dehydrogenase (11β-HSD1), an enzyme attached through an integral N-terminal transmembrane helix to the lipid bilayer and located with its active site within the lumen of the endoplasmic reticulum. Here we report the crystal structure of recombinant guinea pig 11β-HSD1. This variant was determined in complex with NADP at 2.5 Å resolution and crystallized in the presence of detergent and guanidinium hydrochloride. The overall structure of guinea pig 11β-HSD1 shows a clear relationship to other members of the superfamily of short-chain dehydrogenases/reductases but harbors a unique C-terminal helical segment that fulfills three essential functions and accordingly is involved in subunit interactions, contributes to active site architecture, and is necessary for lipid-membrane interactions. The structure provides a model for enzyme-lipid bilayer interactions and suggests a funneling of lipophilic substrates such as steroid hormones from the hydrophobic membrane environment to the enzyme active site.

Glucocorticoid hormones control essential functions such as development, immune response, and metabolism. The primary mode of action is accomplished through binding to intracellular glucocorticoid receptors, which, in combination with other factors, can induce or repress gene transcription. This basic principle is in a tissue-specific manner modulated by the enzyme system 11β-hydroxysteroid dehydrogenase (11β-HSD), which interconverts the steroid C11 carbonyl/β-hydroxyl groups in cortisone and cortisol (in humans) or dehydrocorticosterone and corticosterone (in rodents) (1–4). Free plasma levels of cortisol/corticosterone are low, due to tight plasma protein (corticosteroid-binding globulin) binding, whereas cortisone/dehydrocorticosterone do not bind, resulting in high free levels of this glucocorticoid precursor, to be taken up by target cells. Cortisol/corticosterone bind to the glucocorticoid receptor and can exhibit in this receptor-ligand complex transcriptional activity; in contrast, cortisone/dehydrocorticosterone cannot. Thus 11β-HSD is a central “cellular switch,” which decides whether and how much receptor ligand is available (1–4).

At present, two isozymes (11β-HSD1 and 11β-HSD2) are known, and both act seemingly in a mutually exclusive manner (1, 3). Both are bound through N-terminal transmembrane domains to the endoplasmic reticulum (ER) (1, 3). The active site of 11β-HSD1 is located in the luminal compartment of the ER, whereas 11β-HSD2 faces the cytosolic space. These specific localizations explain why 11β-HSD1 is in most instances an NADPH-dependent cortisone reductase, whereas the type 2 enzyme acts as an NAD+-dependent dehydrogenase of cortisol. The cellular functions of 11β-HSD1 (as a cortisol-producing catalyst) in insulin resistance and metabolic disorders establish this enzyme as a novel pharmacological target in non-insulin-dependent diabetes mellitus, and successful inhibitor developments have been reported (5, 6).

Both isozymes belong to the superfamily of short-chain dehydrogenases/reductases (SDR) (1, 3, 4), a large family of enzymes with well over 3000 primary structures from all forms of life deposited in databases (7, 8). SDR enzymes display a low level of residue identities (typically 10–30%) sharing few conserved sequence motifs, which are necessary for the maintenance of fold and catalysis (8, 9). About 30 three-dimensional structures of this family have been deposited in the PDB, showing a nearly superimposable α/β-folding pattern with a central Rossmann fold for nucleotide cofactor binding (8, 9). However, all enzymes show unique active site cavi- ties, thus explaining the large variability in substrate specificities found in SDR enzymes. Purification and thorough characterization of the membrane-attached 11β-HSD isozymes have been difficult, and no structure determination has been reported yet. We recently described the successful expression and kinetic characterization of transmembrane domain-deleted human and

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The abbreviations used are: 11β-HSD1, 11β-hydroxysteroid dehydrogenase; ER, endoplasmic reticulum; GuHCl, guanidine hydrochloride; SDR, short-chain dehydrogenase/reductase; AADP, 3-amino-2-(hydroxymethyl)propane-1,3-diol; PDB, Protein Data Bank.
guinea pig 11β-HSD1 variants (10, 11). We now present the successful crystallization and structure determination at 2.5 Å of the guinea pig enzyme and discuss the structural implications on enzyme function and membrane attachment.

**EXPERIMENTAL PROCEDURES**

**Purification of Guinea Pig 11β-HSD1**—N-terminally His-tagged, transmembrane domain-deleted 11β-HSD1 was expressed in the *Esherichia coli* expression strain BL21(DE3) as described previously (10). Optimization of protein production and purification conditions has been described in detail elsewhere (11). In summary, the supernatant of the cell lysate was subjected to His bind affinity chromatography followed by incubation overnight in buffer supplemented with 0.5 mM GuHCl (ICC Chemicals, Cincinatti, OH), 0.5% Triton X-100 (Anapoex X-100; Anatrace, Maumee, OH), 50 μM NADP+, and 25 μM arylsulfonamido-thiazole inhibitor BVT.4584 (Biovitrum, Stockholm, Sweden), and a final gel filtration chromatographic step on a Superdex 200 HiLoad 26/60 column (Amersham Biosciences). The protein was concentrated in the final gel filtration chromatographic step on a Superdex 200 HiLoad 26/60 column (Amersham Biosciences). The protein was concentrated to around 10 mg/ml and centrifuged in a Microfuge for 30 min to remove the cell-free supernatant. The resulting electron density figure of merit was improved from 0.34 to 0.76. The resulting electron density maps were of sufficient quality to place most residues of the guinea pig 11β-HSD1 dimer, Clear density for the cofactor was also observed. The model was built manually using O (16), and subsequent rounds of refinement were carried out using REFMAC (17). During the final refinement stages, water molecules were added to the protein model using ARP/wARP (18). The final model contained residues 24–300 from chain A and 24–292 from chain B, 2 NADP and 70 water molecules in the asymmetric unit with 90.3% of residues in the most favored regions of the Ramachandran plot, and 2 residues in the disallowed region. The model has root mean square deviations of 0.015 Å in bond lengths and 1.7 Å in bond angles. The R cryst and R free values of the final model were 19.2 and 26.7%, respectively (Table I). The atomic coordinates have been deposited at the Protein Data Bank with accession code 1XSE.

**RESULTS AND DISCUSSION**

Crystallographic data

| Data collection | Data set | HgCl | HgCN |
|----------------|---------|------|------|
| Resolution (Å) |          |      |      |
| 15.0–2.5       | 20–3.5  | 15.0–2.7 |
| (2.6–2.5)      | (3.6–3.5) | (2.8–2.7) |
| Completeness (%) | 99.5 (97.8) | 97.6 | 99.8 |
|                  | (98.9) | 98.8 |
| I/σ             | 25.3 (3.7) | 21.9 | 36.7 |
| R cryst         | 0.046 (0.272) | 0.122 | 0.062 |
|                  | (0.240) | (0.249) |
| R free          | 0.22 | 0.13 |
| R calc          | 0.59 | 0.73 |
| Phasing power   | 26.8 | 36.7 |
| Number of sites | 2 | 2 |
| Refinement      | R cryst (%) | 0.192 |
|                  | R free (%) | 0.267 |
| Root mean square deviation | Bonds | 0.015 |
|                  | Angles | 1.77 |
|                  | Dihedrals | 8.35 |
| Ramachandran distribution (%) | 90.3% most favorable, 9.1% allowed, 0.4% generously allowed, 0.2% disallowed |
posed role in membrane interactions (discussed below), the C-terminal regions thus fulfill essential roles in subunit interactions, membrane topology, and active site architecture.

At present, primary structures of 11β-HSD1 have been determined from 13 different species, and a pattern of conserved and variable segments of 11β-HSD1s has been established. Two six variable segments (V1–V6) can be defined, and they all cluster together to form parts of the substrate-binding pocket and the dimer interface (Fig. 1). Regions V1 (residues 122–131), V4 (residues 225–236), V5 (residues 259–269), and V6 (residues 280–300) of the second subunit form the substrate-binding pocket, whereas V2 (residues 176–182) form parts of the dimer interface interacting with V3 (residues 198–205) and V6 of the second subunit. The low homology around the substrate-binding pocket is manifested in the different substrate specificities (cortisol/corticosterone, 7α/7β-hydroxycholesterol) and the fact that species-specific inhibitors are frequently found. The guinea pig 11β-HSD1 has an 8-residue extension in the C terminus when compared with the other orthologue sequences determined.

**Structural Features of Guinea Pig 11β-HSD1 and Relationship to the Short-chain Dehydrogenase/Reductase Family**—The closest structural neighbors to guinea pig 11β-HSD1 are SDR enzymes such as porcine carbonyl reductase (PDB accession code 1CYD), trihydroxynaphthalene reductase from *Magnaporthe grisea* (PDB accession code 1D0H), human estradiol 17β-hydroxysteroid dehydrogenase type 1 (PDB accession code 1BHS), and *E. coli* 7α-hydroxysteroid dehydrogenase (PDB accession code 1AHI), showing high similarity (about 2 Å root mean square deviation over 240 residues) in the central scaffold. In particular, the essential sequence motifs of the SDR family are found at homologous positions, such as the N-terminal sequence TGXGXG (positions 17–24), necessary for nucleotide binding, and the active site tetrad consisting of Asn-119, Ser-170, Tyr-183, and Lys-187 (21–24). Major differences between these enzymes are seen in their substrate-binding loop, probably reflecting the differences in substrate specificity, as well as in the previously mentioned C-terminal fragment, reflecting differences in quaternary structure arrangement.

A segment of IRV residues, with Asn constituting an N-linked glycosylation site in 11β-HSD1, is highly conserved in SDR enzymes. Mutational studies on this residue in a related steroid dehydrogenase showed the essential nature for catalysis, and sequence comparisons suggested a homologous role for Asn-207 found in 11β-HSD1 (9). Mutational analysis of this residue in rat 11β-HSD1 indeed revealed that it is essential for activity (25). Later experiments, however, suggested that the glycan moiety itself is not a prerequisite for activity (26). In the guinea pig 11β-HSD1 structure, the conserved Asn-207 is located at the enzyme surface in a loop connecting helix F and strand F, which is consistent with glycan modification in a eukaryotic cellular ER environment. Despite an apparent homology to the conserved Asn residue in the IRVN sequence of SDRs (9), Asn-207 in guinea pig 11β-HSD1 does not connect the active site and the C-terminal part of SDRs; instead, this stabilizing function is fulfilled by Thr-211. Its side-chain OH connects through a water molecule to the OH of Ser-164, located at the enzyme surface in a loop connecting helix F and strand F, which is consistent with glycan modification in a eukaryotic cellular ER environment. Despite an apparent homology to the conserved Asn residue in the IRVN sequence of SDRs (9), Asn-207 in guinea pig 11β-HSD1 does not connect the active site and the C-terminal part of SDRs; instead, this stabilizing function is fulfilled by Thr-211. Its side-chain OH connects through a water molecule to the OH of Ser-164, located in strand E close to the active site, and to the carbonyl of the C-terminal residue Gln-253. Thus the role of Asn-207 in catalysis remains unknown; however, it is possible that Asn-207 is necessary for glycan modification and subsequent folding in the ER of mammalian cells.

Guinea pig 11β-HSD1 contains cysteine residues at sequence positions 75, 213, and 241. Two of these residues, Cys-213 and

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Cys-241, are found in all sequenced 11\(\beta\)-HSD1 variants, whereas only the porcine orthologue shows variation at position 78 (Ser instead of Cys). A disulfide bond involving Cys-78 and Cys-213 has been suggested to be present in the rabbit orthologue (27). This hypothesis is apparently supported by the subcellular localization of the catalytic domain of 11\(\beta\)-HSD1 within the ER lumen, which provides an oxidative environment suitable for disul-
fide bond formation. However, examination of the guinea pig structure raises serious doubts about the possibility of an intramolecular disulfide bond to be formed in 11β-HSD1 variants. Although the distance between the α-carbons of Cys-78 and Cys-213 is about 20 Å, the corresponding distance between the closest cysteine pair, Cys-213 and Cys-241, is almost 10 Å, thus excluding intrasubunit disulfide formation.

Active Site Topography and Catalysis—The general oxidoreductase mechanism of SDRs is reflected in a common active site configuration. The binary 11β-HSD1-NADP⁺ structure fulfills the requirements for a catalytically competent complex. In general, hydride transfer in SDR enzymes is carried through by the C4-H from the S-face of the coenzyme (28), in which Tyr-183 acts as an acid-base catalyst, Lys-187 lowers the pKₐ of the tyrosine hydroxyl, and Ser-170 stabilizes the substrate carbonyl group (28). In addition, the Tyr and Lys residues form together with the 2'-OH of the nicotinamide ribose, a water molecule, and the main-chain carbonyl of the conserved Asn-143 residue, a hydrogen-bonding network, suggesting a proton relay from the active site to the bulk solvent (Fig. 2) (22).

Three regions can be distinguished within the NADP(H)-binding site. Residues surrounding the region forming the cleft for the nicotinamide ring and its ribose are Asn-119, Val-121, Tyr-123, Val-168, Ser-169, Ser-170, Tyr-183, Lys-187, Leu-215, Gly-216, Leu-217, Ile-218, and Ala-22. Residues Leu-215 and Gly-216 that terminate β-strand F (Fig. 1) are at homologous positions of a conserved Pro-Gly motif found in other SDRs and are oriented toward the R-side of the nicotinamide ring. The carbonyl and amide groups of this conserved glycine residue are possibly involved in the initiation of catalysis (29); accordingly, in guinea pig 11β-HSD1, the carbonyl of Gly-193 is oriented toward the C4h of the R-side of NADP. The pyrophosphate and adenine ribose moieties are bound in a cavity built up mainly by the conserved residues Gly-41-Ile-46 and the segment Ile-220-Thr-222. A mostly apolar surface for the adenine ring is created by segments Gly-91-Met-93 and residues His-120, Val-121, and Val-142, whereas the other side of the ring is dominated by the adenine ribose. The 2'-OH phosphate of NADP is bound to Ser-67 and Arg-66, but not to Lys-44, as observed in other NADP(H)-dependent SDRs, such as mouse lung carbonyl reductase (30). Accordingly, NADP(H) specificity versus NAD(H) is achieved through Arg-66. This residue is not present in NAD(H)-dependent SDRs and is also preceded in these enzymes by an Asp residue conferring electrostatic repulsion to a 2'-phosphate (30, 31).

The shape of the substrate-binding pocket is compatible with its function to accommodate cortisol/cortisone and 7β-hydroxycholesterol/7-ketocholesterol. The substrate-accessible void is 640 Å³ and is predominantly lined by non-polar residues (Fig. 2a). To gain insights into the determinants for substrate binding, cortisol was manually docked into the present structure (Fig. 2b). Carbon 11 was fixed within hydride transfer distance to C4 and the C11 hydroxyl group within hydrogen-bonding distance to Ser-170 and Tyr-183. These experiments reveal that cortisol fits well into the active site, provided that a side-chain movement of Tyr-123 occurs for accommodation of the substrate. Residues in α1, the nicotinamide ring of NADP, and residues in variable segment 1 constitute the lower part of the pocket, whereas Ile-180 and Leu-217 are crucial for the middle part. The upper part, which according to our modeling should accommodate the A-ring of the substrate, offers several possible hydrogen bond donors to the 3-keto group of cortisol, e.g. the side-chain hydroxyls of Tyr-177 and Tyr-231 and the backbone amide nitrogen of Leu-217. Notably, Ser-280 and Tyr-284 of the second subunit are lining the upper part of the substrate-binding pocket. The pocket stretches further up at this end, and the substrate-binding loop, the loop connecting βF and α2, together with α2 of the second subunit form a possible entrance.

Orientation of 11β-HSD1 with Respect to the Lipid Membrane—The location of the N termini together with the amphipathic nature of the C-terminal helices in the 11β-HSD1 dimer suggest a possible orientation of the molecule with respect to the membrane surface (Figs. 3 and 4). The two C-terminal helical segments from the dimer form a non-polar plateau (−3000 Å²), which is encircled by a ring of positively charged residues (Fig. 3). This arrangement suggests that the plateau is located in the non-polar center of the membrane with the charged residues forming salt bridges with the displaced phospholipid and sulfolipid head groups. This model characterizes monotopic membrane proteins, and such a non-polar surface contact formed at a dimer interface has been previously observed for squalene cyclase (32) and prostaglandin-H2-synthase (33).

A possible entrance to the substrate-binding pocket is formed by the substrate-binding loop, the loop connecting βF, and helices α2 and α2 of the second subunit. With the suggested membrane orientation, this entrance makes the catalytic site accessible for lipophilic substrates such as cortisol or 7β-hydroxycholesterol, enriched in the lipid membrane. Accordingly, this model intuitively suggests a funneling of hydrophobic steroids from the membrane into the active site cleft. This hypothesis further explains the discrepancies between efficient steroid conversion at low concentrations and the somewhat higher apparent Kₘ values observed in vitro (10, 34).

Taken together, this study provides detailed insight into the structural features and active site architecture of a physiologically important hormone-activating enzyme and drug target. Furthermore, the observed hydrophobic surface and the proposed membrane association might constitute a general model for lipid interactions of similar membrane-bound SDR enzymes such as 17β-HSD types 2 and 3 or enzymes involved in retinoic acid metabolism such as retinol dehydrogenases.

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