Crystal Structure of a Recombinant Anti-estradiol Fab Fragment in Complex with 17β-Estradiol*

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The crystal structure of a Fab fragment of an anti-17β-estradiol antibody 57-2 was determined in the absence and presence of the steroid ligand, 17β-estradiol (E2), at 2.5 and 2.15 Å resolutions, respectively. The antibody binds the steroid in a deep hydrophobic pocket formed at the interface between the variable domains. No major structural rearrangements take place upon ligand binding; however, a large part of the heavy chain variable domain near the binding pocket is unusually flexible and is partly stabilized when the steroid is bound. The nonpolar steroid skeleton of E2 is recognized by a number of hydrophobic interactions, whereas the two hydroxyl groups of E2 are hydrogen-bonded to the protein. Especially, the 17-hydroxy group of E2 is recognized by an intricate hydrogen bonding network in which the 3- and 17-hydroxyl groups are hydrogen-bonded to the protein. The CDRL3 loop plays a prominent role in ligand binding. All the complementarity-determining regions of the light chain make direct contacts with the steroid, even CDRL2, which is rarely directly involved in the binding of hapten.

The steroid 17β-estradiol (E2)† is the major female sex hormone, and it has disseminated roles in a wide range of reproductive and nonreproductive physiological processes in different organs. E2 is the cell proliferation-promoting steroid hormone during the menstrual cycle, and it has known functions in bone metabolism and in the cardiovascular and central nervous systems. E2 is the most potent member of the family of estrogens, including estrone and estriol, and it binds tighter to hormone-dependent disorders; for example, supplementary E2 administered to postmenopausal women relieves postmenopausal symptoms, reverses urogenital atrophy, and increases bone mass. On the other hand, increased serum E2 levels have been linked with malignant/premalignant diseases such as breast cancer (3) and endometriosis (4).

Immunoassays are routinely used to measure serum E2 levels. However, the generation of monoclonal antibodies with high enough affinity and specificity for steroid hormones has proven to be very difficult with the conventional hybridoma techniques. The conserved hydrophobic steroid skeleton of E2 with only two polar heavy atoms (3- and 17-hydroxyl groups) seems to be a particularly challenging target for the murine immune system, and the current E2 assays are based on polyclonal immunoglobulins from rabbit serum. However, the polyclonal antibodies have disadvantages compared with monoclonals. The most severe problem is a large batch-to-batch variation, and therefore the E2 assays require a laborious optimization procedure with each new batch of polyclonals. Hence, a monoclonal, recombinant, high affinity/specificity anti-E2-antibody would be very desirable and could be of great potential value in clinical assays.

The monoclonal antibody 57-2 has been raised in mice against the E2 conjugated to bovine serum albumin, and the genes encoding its antigen binding regions have previously been cloned in our laboratory (5). The antibody has a moderately high affinity for E2 (Kₐ = 5 × 10⁸) and a good selectivity between different estrogens. Unfortunately, the E2 affinity is not high enough to be useful in clinical assays, and the antibody also cross-reacts with testosterone with a substantially high affinity (Kₐ(testosterone)/Kₐ(E2) = 37%). Molecular modeling was previously used to predict how E2 binds to the 57-2 antibody (6), but in order to obtain structural information on the nature of the E2-Fab interactions with improved accuracy, we have recently crystallized the 57-2 Fab in the absence and presence of 17β-estradiol (7).

Currently, there are few steroid antibody complex crystal structures available in the Protein Data Bank (Research Collaboratory for Structural Bioinformatics, Rutgers University) (8): a progesterone (9), two digoxin-Fab structures (10, 11), and crystal structures of a Fv fragment in complex with estrone-2-glucuronide and estriol-3-glucuronide (12). In this study, we report the crystal structures of the Fab fragment of the antibody 57-2 in the absence and presence of the antigen 17β-estradiol at 2.5 and 2.15 Å, respectively. The high resolution crystal structures provide detailed information of the complex nature of the E2-antibody interactions and explain the previously characterized binding properties of the antibody, especially its excellent selectivity between different estrogens and high cross-reactivity with testosterone. There are some unusual features in the antibody; a large region of the combining...
Crystal Structure of a Fab-Estradiol Complex

The detailed description of the protein expression, purification, and crystallization protocol of the molecule is described elsewhere (7). Briefly, the Fab fragment was (i) overexpressed into periplasmic Escherichia coli and (ii) purified by tandem cation exchange chromatography followed by a second cation exchange chromatography. (iii) The purified protein was desalted and concentrated to ~30 mg/ml, and (iv) crystals were obtained at 276 K using sitting drop vapor diffusion methods with reservoir solutions containing 5% polyethylene glycol 8000 in 100 mM Tris-HCl buffer in high pH (9.0 – 9.5) as the precipitant. Crystals of the Fab-steroid complex were produced by co-crystallization in the presence of 17β-estradiol and cross-seeding with the apo-form crystals. The crystals were flash-cooled using 25% glycerol as the cryoprotectant in a 100 K nitrogen gas stream, and x-ray data were collected with a Rigaku Raxis II diffractometer operated at 50 kV and 180 mA using copper Kα (λ = 1.5418 Å) radiation; data were integrated and scaled with the software DENZO and SCALEPACK (15). The calculated Matthews coefficient (14) was consistent with one Fab fragment in the asymmetric unit.

The Fab structures were solved by molecular replacement methods (15, 16). The novel software CNS (17) was used in all molecular replacement and refinement work, and the software O (18) was used for displaying electron density maps and for model building. Before any structure solution was attempted, 7–10% of the reflections were randomly set aside and used only for calculations of the cross-validated error function Rfree (19) but never in refinement or map calculations. A hybrid Fab fragment model that was used as a template in the molecular replacement studies was constructed from the Protein Data Bank files 1TET (variable domain of heavy chain; Ref. 20) and 1FDL (variable domain of light chain; Ref. 21). The rotation and translation function produced a very clear solution in the correct space group. After a rigid body refinement followed by a torsion angle dynamics simulated annealing (22) run, the Rfree dropped to 37%, and initial maps (resolution 50 – 2.7 Å) were calculated. When electron density maps were inspected using a graphics work station, a clear E2 electron density (>5 σ over the mean density in (Fobs – Fcalc)/exp(-1.0*σ(Fobs)) maps) was seen in the maps generated with x-ray data collected from crystals obtained with microseeding in the presence of E2.

Further refinement work proceeded smoothly as an iterative process between interactive graphics work with O and refinement with CNS. All x-ray data to 2.15 Å in the E2 complex structure and all data to 2.5 Å resolution in the apo structure were used in all subsequent refinements without a σ cut-off. An anisotropic B-factor correction was used, and a bulk solvent correction was applied to model the low resolution reflections. The refinement method varied between the deepest descent approach and simulated annealing torsion angle dynamics (23) methods using the novel maximum likelihood function (22) as the refinement target. The simulated annealing-torsion angle refinement was always utilized when a drop in Rfree justified its use. The calculated 2Fobs – Fcalc and Fobs – Fcalc maps used in model building were always oA (24) weighted.

When the R-factor had dropped below 30% and missing side chains had been included in the model, we began adding water molecules to the structure. The criteria for water addition was as follows. The waters were only added to the model in places where Fobs – Fcalc electron density maps had a peak value 3 σ over the mean with reasonable hydrogen bonding geometry to the protein. Also, after adding the water molecule to the model, the 2Fobs – Fcalc maps were required to show a peak height of 1.5 σ in the putative water molecule position and a real space correlation coefficient greater than 0.50. B-factors were released at the same time when water building was started; the use of individual B-factor refinement versus grouped B-factor refinement was again validated with Rfree. A number of simulated annealing omit maps (25) were calculated in regions of ill defined electron density, and the models were modified accordingly. The steroid was built into the maps as a rigid molecule, and the coordinates and potential parameters were obtained from the Hetero-Compound Information Center (Uppsala, Sweden) (Web site: alpha2.bmc.uu.se/hicup) (26). Finally, the molecule was checked against pathologies with the Whatcheck program in the software package WHAT IF (27), which led to only minor changes in the structure. The program InsightII (Molecular Simulations, San Diego, CA) was used for molecular modeling, as two candidates of other steroids were obtained from the Cambridge Structural Data Base (28). The figures in this work were prepared with Molscript (29) and Raster3D (30).

RESULTS

Overall Structure—The three-dimensional crystal structures of 57-2 Fab fragment in the absence (apo) and presence (holo) of 17β-estradiol (Fig. 1) were determined to 2.5- and 2.15-Å resolution, respectively. The crystals belong to space group P212121 with one monomer in the asymmetric unit; the crystal and refinement data are presented in Table I. The amino acid sequence of the 57-2 Fab and residue numbering following the Kabat numbering scheme (31) is shown in Fig. 2. The electron density maps were well defined in most parts of the structures, and immunoglobulin fold models with good stereochemistry (Table I) could be built without difficulties. There are three chain breaks in the loop regions in the heavy chains of both models due to poor local electron density. In the holo structure, the regions not built include residues H29 and H30 in the CDR1 of heavy chain, H75 in the loop next to the heavy chain CDR1, and two C-terminal amino acid L214 were built in the light chains of both structures. The apo and holo Fabs have identical packing environments. None of the CDR loops is involved in direct hydrogen bonding interactions, and the Fabs are in very similar conformational environments with the elbow angles between the variable and constant domains about 161°, a value that is in the range commonly observed for Fab structures (32).

CDR Loop Conformations—Four out of the total six CDR loops (Fig. 2) in both structures could be classified to canonical
classes according to the definitions by Chothia and Lesk (33). The CDR1 belongs to canonical class 2, and the CDR2 and CDR3 loops are both of class 1. The heavy chain CDR2 was judged to belong to the canonical class 2. Because of the chain break in CDR1, it was not possible to define the canonical type of this loop. Due to the high diversity of the apex of CRDH3, a comprehensive canonical classification scheme has only been introduced for the stem of the loop (34–36). In 57-2, the stem of CRDH3 adopts a nonkinked conformation probably because the loop lacks both Arg-H94 and Asp-H101, which typically form a conserved salt bridge in the kinked-type loop.

Recognition of Ligand—Once the estradiol-Fab co-crystallization procedure was established (7), the bound steroid could be unambiguously detected in the electron density maps (Fig. 4). As shown in Figs. 3–5, E2 is bound in a deep pocket formed between the heavy and light chain variable domains (VH and VL). The depth of the cleft is ∼8 Å, and the width and length are 5 and 11 Å, respectively. The complex is oriented parallel to the interface between VH and VL. The D-ring of the steroid penetrates deep into the cleft so that the 17-OH of E2 lies next to the bottom of the binding pocket, whereas the A-ring is located near the mouth of the pocket. The D-ring lies between the stem of CDRH1, contacts the steroid directly. His-L89. Each of these three polar side chains forms additional hydrogen bonds to surrounding residues. The 3-OH group of E2, in turn, forms a hydrogen bond with the carbonyl oxygen of Gly-H97 in CRDH3.

A detailed description of the interactions between the antibody and E2 is presented in Table II and in Fig. 5. The steroid is sandwiched between the side chains of three aromatic residues in the walls of the binding pocket; the α-side (Fig. 1) of the steroid is in contact with Tyr-L49 and Phe-L91, and the β-side is in contact with Trp-H95. The indole side chain of the CRDH3 residue Trp-H95 protrudes between the steroid and CRDH1 loop and is alone responsible for the majority of the contacts that the antibody makes with the β-side of E2. Glycines H96 and H97 and Ala-H101 in CRDH3 interact with the A-ring of E2. CRDH3 has a prominent role in the binding of the ligand; this loop accounts for 45% of the antibody’s solvent-accessible surface that is buried upon the ligand binding. The D-ring end of the steroid is in contact with Trp-L96 from CRDH3, and the bottom of the binding cleft is formed by Gln-H35, Tyr-L36, and His-L89. Only one non-CDRH3 heavy chain residue, Gln-H35 in the stem of CRDH1, contacts the steroid directly.

The two hydroxyl groups of E2 are hydrogen-bonded to the antibody (Fig. 5). The 17-OH of E2 is recognized by an intricate hydrogen bond network (Fig. 6, a and b). In the heart of this network, there is a four-center hydrogen bond between the 17-OH and the side chains of the residues: Gln-H35, Tyr-L36, and His-L89. Each of these three polar side chains forms additional hydrogen bonds to surrounding residues. The 3-OH group of E2, in turn, forms a hydrogen bond with the carbonyl oxygen of Gly-H97 in CRDH3.

Comparison of Apo and Complex Structures—There are no major structural differences between the apo and the holo structures. We could superimpose 419 Cα atoms of the two structures with a root mean square deviation of 0.41 Å, and the difference in the relative domain association angle between VH and VL in the two structures is only 0.45°. The B-factors of the residues (Trp-H95 to Trp-H101) in the CRDH3 loop refined to much higher average values in the apo structure (58.6 Å²) than in the holo structure (27.0 Å²), and there is a slight movement of this loop (root mean square deviation of 0.3 Å when six Cα

| Data collection and refinement statistics |
|-----------------------------------------|
| **Crystal** | **Apo** | **Complex** |
| Space group | P2₁,2,2 | P2₁,2,2 |
| Unit cell dimensions (a, b, c) (Å) | 45.3, 64.5, 165.7 | 48.2, 64.5, 165.3 |
| Unique/Measured reflections | 17081/16612 | 25614/131703 |
| Resolution (Å) | 50.2–5 | 50–1.21 |
| Rmerge (%) | 5.2 (7.8) | 5.1 (13.5) |
| Completeness (%) | 91.8 (72.7) | 88.7 (71.9) |
| Refinement |
| Atoms per molecule | 3379 | 3587 |
| Number of water molecules | 163 | 354 |
| Resolution (Å) | 50.2–5 | 50–1.21 |
| Reflections used | 17,081 | 25,614 |
| R-factor (%) | 20.1 (25.8) | 26.3 (30.2) |
| Free R factor (%) | 25.2 (34.4) | 0.006 |
| r.m.s. deviations, bond angle (°) | 1.3 | 1.4 |
| Average B value (Å²) | 33.2 | 28.8 |
| Residues within allowed regions (%) | 99.1 | 99.6 |

* For the 2.59–2.5 Å resolution shell.
* For the 2.25–2.15 Å resolution shell.
* For the 2.23–2.15 Å resolution shell.
* Root mean square deviations are deviations from the Engh and Huber parameters (53).
atoms were superimposed) upon ligand binding (Fig. 5). The orientations of the residues Tyr-L49 and Asp-L56 are different between the two structures (Fig. 5). These residues are located on both sides of the CDRH3 loop, and their side chains are hydrogen-bonded to CDRH3 residue Asn-H98 in the complex structure. This arrangement could not be detected in the apo structure. In the apo structure, there are two water molecules in the binding site; one is located at the bottom of the binding site close to the position occupied by the 17-OH of E2 in the holo structure, and the other is situated higher in the binding pocket and is hydrogen-bonded to the first one.

Molecular Modeling—The interactions between the antibody with two nonnatural E2 analogs, 17α-estradiol and 17-deoxyestradiol, as well as with natural estrogens estrone (E1) and estriol (E3) were studied with a graphics work station by superimposing these steroids on E2 in the holo structure using the carbon atoms of the steroid skeleton (Fig. 7). The two nonnatural analogs are otherwise identical with E2, but in 17α-estradiol the 17-OH group is oriented to the opposite side of the steroid skeleton than in 17β-estradiol, and 17-deoxyestradiol lacks the 17-OH group (Fig. 1). The superimposition of 17α-estradiol on E2 shows that the 17-OH of 17α-estradiol is too far to hydrogen-bond to Gln-H35 and Tyr-L36 (distances of 5.3 and 4 Å, respectively), and the hydrogen bond-
TABLE II

| Steroid atom | Fab residue | Fab atom |
|--------------|-------------|----------|
| C1           | Tyr-L49     | C81, C2, C3, C4, C52 |
| C2           | Gly-H97     | C2, C3, C7 |
| C3           | Gly-H97     | N         |
| C6           | Gly-H97     | O         |
| C7           | Phe-L91     | C2, C52, C3 |
| C8           | Ser-H99     | C51, C52, C52 |
| C9           | Ser-H99     | N         |
| C10          | Leu-L46     | C62       |
| C11          | Ser-H99     | O         |
| C12          | Ala-H101    | C8        |
| C13          | Tyr-L49     | C8        |
| C14          | Ala-L34     | C8        |
| C15          | Tyr-L49     | C8        |
| C16          | Ala-L34     | C8        |
| C17          | Tyr-L36     | C3        |
| C18          | His-L99     | N3        |
| C19          | Gln-H35     | N2        |
| C20          | His-L99     | N2        |
| C21          | His-L99     | N3        |
| C22          | Ser-L95     | C8, C52, C52 |
| C23          | Trp-L95     | C51, C52, C52 |

The antibody 57-2 recognizes a hydrophobic hapten 17β-estradiol by burying it in a deep nonpolar cleft. The antibodies that bind small molecules often have long forms of some of the V-gene-encoded CDRs (especially CDRH2 and CDR1) (37). These loops protrude out into the solvent and thus promote the generation of pocket-like binding sites that are necessary for recognition of small ligands with limited surface areas. In anti-estriadiol antibody 57-2, all of the CDRs are of the short type, and therefore the binding pocket is formed entirely in the interface between the VH and VL domains.

A large part of the VH domain near the binding site seems to be flexible as judged by poor electron density and B-factors refined to high values. This labile region comprises most of CDRH1, residues H72–H76 in the loop next to CDRs (Fig. 3) and the N-terminal residues H1–H3, in both structures. In the apo structure, it also includes the apex of the CDRH3 loop. The CDRH1 loop that connects the two β-sheets of the VH domain is 7 amino acids long and, according to the definitions by Chothia and Lesk (33), should belong to canonical class 1. These types of CDRH1 loops are normally firmly anchored to the hydrophobic core of the VH domain through buried, bulky, hydrophobic, and/or aromatic residues in positions H27 and H29. These residues are found also in 57-2 (Tyr-H27 and Phe-H29), but they are located in the region of ill defined electron density and are apparently flexible. The unusual flexibility of CDRH1 in 57-2 structure is probably due to an aspartate in the position H26 in the beginning of the CDRH1. This position is generally occupied by a highly conserved glycine that adopts a backbone conformation not allowed for other residue types. Gly-H26 has been defined as one of the key residues for CDRH1 conformation (33, 38). Based on the information from static crystal structures, it is quite difficult to understand the functional role of the apparent flexibility of CDRH1. This loop is disordered both in the presence and absence of the ligand. Because it makes no direct contacts with the steroid higher structural variability might be tolerated. Only one other antibody structure with an Asp in position H26 is currently available from the Protein Data Bank (8): in a low resolution structure of a ternary complex of human chorionic gonadotropin hormone with two antibody Fv fragments (Protein Data Bank code 1QFW; resolution 3.5 Å (39)), the CDRH1 of one of the Fvs is in a conformation that falls outside the canonical classes. In the Fab 57-2 crystal structures, the apparent mobility of the loop between residues H72–H76 and the N terminus is probably due to the flexibility of the neighboring CDRH1. The flexibility of the CDRH3 in the apo structure, in turn, can be largely due to the presence of two consecutive glycine residues (H96 and H97) in the apex of the loop. In the E2-Fab complex structure, CDRH3 is well defined and has low B-factors. Thus, the ligand binding stabilizes CDRH3 that is directly in contact with the steroid.

The recognition of 17β-estradiol by the 57-2 antibody is based on shape and chemical complementarity. The fit between the molecular surfaces of the ligand and the protein is tight for most parts, but the bulkiness of the aromatic residues forming a large part of the walls of the binding pocket seems to limit the shape complementarity. The space between the ligand and surrounding residues is, however, too small to accommodate any water molecules. The high chemical complementarity between the binding pocket and the antigen compensates the potential limitations in the shape complementarity. The two hydroxyl groups of E2 are both in a polar environment and recognized by hydrogen bonds, whereas the nonpolar steroid skeleton is contacted by a number of hydrophobic interactions.
made mostly by the aromatic residues, including two tyrosines and two tryptophans. Aromatic residues (particularly tyrosine and tryptophan) are abundant in the binding sites of antibodies (40, 41) and seem to be especially common in the binding pockets of the antibodies recognizing small hydrophobic haptons (12, 42–44). These antibodies must generally be able to provide a nonpolar binding site in a polar aqueous environment, and the physico-chemical properties of the aromatic residues are apparently favorable for this purpose. In antibody DB3, raised against progesterone, there are several aromatic residues in the binding site but also a gate-keeping tryptophan residue that excludes water from the interior of the binding pocket in the absence of ligand (42). In antibody 57-2, this type of conformational change-based water exclusion mechanism is
not observed, and two water molecules are seen in the binding pocket in the apo structure.

The antibody recognizes the only polar parts of 17β-estradiol, the 3- and 17-hydroxyl groups, by a total of four hydrogen bonds. The 17-hydroxyl of E2 that lies deep in the binding pocket is directly hydrogen-bonded to three side chains of the antibody in a rare four-center hydrogen bond (45) (Figs. 5 and 6, a and b). Interestingly, one of the three hydrogen bond-forming residues, Tyr-L36, is usually completely buried in the variable domain interface and is rarely involved in contacts with the antigen in the known structures of antibody-antigen complexes (e.g., in only one of the 26 structures studied by MacCallum et al. (46)). The interatomic distances and the relative orientations between the donor and acceptor groups within the four-center hydrogen bond are favorable for hydrogen bonding (Fig. 6a), and there are no competing water molecules in the nonaqueous environment at the bottom of the binding pocket.

This hydrogen bonding arrangement has a substantial impact on the binding affinity as well as on the specificity of ligand recognition. The information of the antibody-E2 interactions from the crystal structure combined with molecular modeling provides explanations for the previously measured low cross-reactivities of the antibody 57-2 with two nonnatural E2 analogs 17α-estradiol and 17-deoxysteradial as well as with natural estrogens E1 and E3 (6). Simple molecular modeling suggests that the four-center hydrogen bonding geometry is very sensitive to substitutions at the position C17 in the steroid skeleton (Fig. 1). The 17α-estradiol and 17-deoxysteradial cannot form the four-center hydrogen bond and are, therefore, recognized with over 4000-fold lower affinity than E2 (6). Estrone, which is recognized with an about 2200-fold decreased affinity, seems to be able to make only a single hydrogen bond with the antibody at position C17. Estriol has the potential to form a similar hydrogen bond network as E2 but has an altered D-ring conformation and an extra hydroxyl group at C16, which presumably results in a 555-fold reduced affinity.

The structure of the Fab-E2 complex shows (Fig. 5) that the 3-OH of E2 is partially solvent-exposed, and the hydrogen bond between it and the carbonyl group of Gly-H97 is susceptible to competition by water molecules. Gly-H97 is one of the two consecutive glycines in the tip of CDRH3 and the flexibility of this loop might further destabilize this hydrogen bond. Modifications at the 3-OH group of E2 have a much smaller influence on the binding affinity than those at 17-OH. For example, the affinity of 17β-estradiol-3-methyl ether, which has an additional methyl group attached to the 3-oxygen, is only 16-fold lower than that of 17β-estradiol (6). Unlike 3-OH, the etherified oxygen in the position C3 of this derivative cannot act as a proton donor in hydrogen bonding. The location of 3-OH at the mouth of the binding site also explains the capability of the antibody to recognize E2 derivatives with additional chemical groups attached to the 3-oxygen. Even bulky sulfate and glucuronide moieties can be attached to position 3-oxygen, and the binding is not completely blocked ($K_e(E2-3-sodium-sulfate)/K_e(E2) = 7.1\%$ and $K_e(E2-3-glucuronide)/K_e(E2) = 23\%$).

The partially solvent-exposed A-ring of E2 is recognized less specifically than the completely buried D-ring. This is indicated by a lower number of pairwise atomic contacts between the antibody and the atoms in the A-ring (20 contacts) than those in the D-ring of the steroid (28 contacts) (Table II). The lower specificity for the A-ring explains the antibody 57-2’s high cross-reactivity with testosterone. The structure of testosterone one differs from E2 in its A-ring, which is a nonaromatic six-carbon ring instead of a planar aromatic ring of E2, and there is a keto group attached to C3 instead of a hydroxyl group. In addition, testosterone has an extra methyl group at position C10. The CDRH3 loop is responsible for most of the interactions that the antibody makes with the A-ring of the steroid. The potential flexibility of the CDRH3, due to the presence of the two consecutive glycines at the tip of the loop, can also aid the antibody to adapt to the differences in the A-ring of the steroids.

The light chain has an important role in the recognition of the ligand in antibody 57-2. Over half (52%) of the solvent-accessible surface buried upon the ligand binding consists of light chain residues. Even CDRL2, which is rarely in direct contact with the hapten in small molecule recognizing antibodies (32, 47), makes contacts with E2. The residue Tyr-L49 makes many contacts with the A- and B-rings of E2 (Table II), and the side chain of Leu-L46 contacts the C-ring of E2 at the bottom of the binding pocket. In addition, Tyr-L49 and residue Asp-L56 in the stem of CDRL2 seem to participate in the stabilization of CDRH3 in the holo structure by hydrogen bonding with Asn-H98 in the tip of CDRH3 (Fig. 5).

Two structures with very homologous light chains to that of 57-2 are available in the Protein Data Bank, namely 1FDL (21) and 1WEJ (48). The Vl of 1FDL contains seven different amino acids as compared with that of 57-2, and six of them are located in the CDRs. The Vj1 of 1WEJ and 57-2, in turn, differ only in 3 residues, and just one of these, at position L89, is a CDR residue. All of these antibodies seem to utilize the same Vl germ line gene (locus MMIG27; accession V00778 (49) and Jx1 gene (50). The antibodies 1WEJ and 1FDL recognize protein antigens, and the light chains are involved in direct interactions with the bound antigen. The presence of such a similar light chain in a steroid binding antibody illustrates the capability of the immunoglobulins to recognize a wide variety of targets with limited structural changes.

We have previously modified Fab 57-2 by means of protein engineering in several studies, and mutant antibodies showing improved affinity and specificity have been obtained (6, 51, 52). The solid information from the crystal structures described in this work will provide a good basis for the examination of the mechanisms of the mutations and could also aid in the designing of new experiments to further improve the binding properties of the antibody. The crystal structure of the E2-Fab complex presented in this work represents one of the few examples where atomic resolution information of the steroid-antibody interactions has been obtained.

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