Affinity Labeling of Rat Glutathione S-Transferase Isozyme 1-1 by 17β-Iodoacetoxy-estradiol-3-sulfate*

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Rat liver glutathione S-transferase, isozyme 1-1, catalyzes the glutathione-dependent isomerization of Δ5-androstene-3,17-dione and also binds steroid sulfates at a nonsubstrate inhibitory steroid site. 17β-Iodoacetoxy-estradiol-3-sulfate, a reactive steroid analogue, produces a time-dependent inactivation of this glutathione S-transferase to a limit of 60% residual activity. The rate constant for inactivation (kobs) exhibits a nonlinear dependence on reagent concentration with K_i = 71 μM and kmax = 0.0133 min⁻¹. Complete protection against inactivation is provided by 17β-estradiol-3,17-disulfate, whereas Δ5-androstene-3,17-dione and S-methylglutathione have little effect on kobs. These results indicate that 17β-iodoacetoxy-estradiol-3-sulfate reacts as an affinity label of the nonsubstrate steroid site rather than of the substrate sites occupied by Δ5-androstene-3,17-dione or glutathione. Loss of activity occurs concomitant with incorporation of about 1 mol 14C-labeled reagent/mol enzyme dimer when the enzyme is maximally inactivated. Isolation of the labeled peptide from the chymotryptic digest shows that Cys17 is the only enzymatic amino acid modified. Covalent modification of Cys17 by 17β-iodoacetoxy-estradiol-3-sulfate on subunit A prevents reaction of the steroid analogue with subunit B. These results and examination of the crystal structure of the enzyme suggest that the interaction between the two subunits of glutathione S-transferase 1-1, and the electrostatic attraction between the 3-sulfate of the reagent and Arg50 of subunit B, are important in binding steroid sulfates at the nonsubstrate steroid binding site and in determining the specificity of this affinity label.

Glutathione S-transferases (GST)1 (EC 2.5.1.18) constitute a family of detoxification enzymes that are involved in the metabolism of endogenous and xenobiotic compounds (1–4). They catalyze the conjugation reaction of glutathione to a wide variety of electrophilic substrates. These conjugation products are more water-soluble than the xenobiotic substrates, and they can be further degraded or transported out of the cell. Glutathione S-transferases have been found in elevated levels within cancerous tumors and have been implicated in the development of resistance to anti-cancer drugs (5). The cytosolic enzymes are now grouped into seven classes and within a particular class they can exist as either homo- or heterodimers (1). There are crystal structures to represent most of the classes (6–12). Each subunit of the dimer contains a glutathione-binding site and a xenobiotic site that can accommodate a wide variety of compounds.

Isozyme 1-1,2 a member of the α class, efficiently catalyzes the isomerization reaction of Δ5-androstene-3,17-dione to Δ5-androstene-3,17-dione, which it binds at the substrate steroid site (13). In addition to this site, isozyme 1-1 also has a nonsubstrate steroid binding site that is located in the cleft between the two subunits (14, 15). This site has been proposed to fulfill a transport function (5) or to act in controlling levels of steroids in target organs (16). The nonsubstrate site has a preference for steroid sulfates, which is illustrated by the more potent inhibitory effect of 17β-estradiol-3,17-disulfate as compared with that of 17β-estradiol. However, previous work in this laboratory (aimed at locating the nonsubstrate site) used the affinity label 3β-iodoacetoxydehydrosandrosterone (3β-IDA) (shown in Fig. 1), which is structurally related both to substrates of the enzyme, such as Δ5-androstene-3,17-dione, and to inhibitors of the enzyme, such as Δ5-androstene-3β,17β-diol disulfate and 17β-estradiol-3,17-disulfate. The 3β-IDA modified Cys17 and Cys111 equally with an incorporation of 1 mol of reagent/mol enzyme subunit; analysis of molecular models suggested that the binding site of 3β-IDA is located in the cleft between the subunits (15). Based on the previous data, we have now designed a more specific affinity label for the nonsubstrate steroid site: 17β-iodoacetoxy-estradiol-3-sulfate (17β-IES). This new compound features the negatively charged sulfate that should enhance and direct its binding and a reactive iodoacetoxy group at a position at the opposite end of the molecule from that of 3β-IDA (Fig. 1). The iodide can be displaced from the iodoacetoxy group by nucleophilic attack by the side chains of several amino acids including Cys, Asp, Lys, Met, and His (17). In this paper, we demonstrate that this affinity label reacts specifically with Cys17 at a single subunit of the enzyme dimer. Molecular modeling studies support the location of the nonsubstrate binding site within the cleft and the contribution of the sulfate moiety in orienting the ligand within the cleft. A preliminary version of this work has been presented (18).

EXPERIMENTAL PROCEDURES

Materials—Frozen Harlan Sprague-Dawley rat livers were purchased from Pel Freez Biologicals, glutathione, S-hexylglutathione, S-hexylglutathione-Sephrose, S-methylglutathione, Sephadex G-50, iodoacetic acid, α-chymotrypsin, 17β-estradiol-3,17-disulfate, 17β-estradiol-3-sulfate, N,N′-dicyclohexylcarbodimide, and 1-chloro-2,4-dinitrobenzene were purchased from Sigma. 17β-Estradiol-17-sulfate and Δ5-androstene-3,17-dione were provided by Steraloids, Inc., and [1-14C]iodoacetic acid was purchased from Moravek Biochemicals. Bio-

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1 The abbreviations used are: GST, glutathione S-transferase; 3β-IDA, 3β-(iodoacetoxy)dehydrosandrosterone; 17β-IES, 17β-iodoacetoxy-estradiol-3-sulfate; HPLC, high pressure liquid chromatography.

2 Glutathione S-transferase, isozyme 1-1, is designated as the rGSTA1,2 isozyme in the nomenclature of Hayes and Pulford (5).
Fig. 1. Steroids that bind to glutathione S-transferase, isozyme 1-1. 3β-
Androstene-3,17-dione (substrate), 17β-
estradiol-3,17-disulfate (reversible inhib-
itor), 3β-(iodoacetoxy)dehydrossoandros-
terone (affinity label), and 17β-iodoace-
toxy-estradiol-3-sulfate (affinity label) are shown.

Rad Laboratories provided Protein Assay Dye Reagent, and Liquiscent
was purchased from National Diagnostics.

Enzyme Preparation—Glutathione S-transferase isozyme 1-1 was
purified from rat livers using affinity column chromatography on S-
heptylglutathione-Sepharose (19). Values of ε270 nm = 22,000 M−1 cm−1
(20) and molecular weight of 25,500 per subunit (2) for GST 1-1 were used to
calculate the enzyme concentration.

Synthesis of 17β-Iodoacetoxy-estradiol-3-sulfate—17β-IES was syn-
thesized from 17β-estradiol-3-sulfate and iodoacetic acid by procedures
based on the method of Pons et al. (21). One molar equivalent of
17β-estradiol-3-sulfate, 1.1 molar equivalents of iodoacetic acid, and 2
molar equivalents of dicyclohexylcarbodiimide were combined in 15 ml
of cellophane. (For the radioactively labeled compound before addition to
the reaction mixture, 125 µCi of radioactive iodoacetic acid was added
to 0.83 mmol of unlabeled iodoacetic acid in a total of 5 ml.) The reaction
was initiated by the addition of a catalytic amount of pyridine (250 µl),
and the reaction mixture was allowed to stir at room temperature for
1.5 h. The reaction was stopped by the addition of 3 ml of distilled
water, and the mixture was centrifuged to remove the insoluble dicy-
clohexylurea. The organic layer, containing 17β-IES, was lyophilized.
The product was resuspended in 100 µl of acetonitrile and was brought
to a final volume of 1 ml by the addition of distilled water.

The 17β-IES was purified by HPLC using a Varian 5000LC equipped
with a Vydac C$_{18}$ column (1 × 25 cm) and a UV-100 detector. The solvent
system used was H$_2$O (Solvent A) and acetonitrile (Solvent B).
The column was equilibrated with solvent A containing 10% solvent B.
After 10 min at 10% solvent B, a linear gradient was run to 100% B in
90 min at a flow rate of 1 ml/min. The effluent was monitored at 275 nm
and 17β-IES elut at ~28 min. For comparison, the starting material,
17β-estradiol-3-sulfate, elutes at ~23 min.

For the radioactively labeled compound, the specific radioactivity
was 2.17 × 10$^{11}$ cpm/mol. The product has a UV absorption spectrum with a maximum at 260 nm and a shoulder at 270 nm. The extinction
coefficient at 260 nm was measured to be 1810 M$^{-1}$ cm$^{-1}$, with the
concentration determined from the specific radioactivity.

Enzymatic Assays—Enzymatic activity was measured by using a
Hewlett Packard 8453 UV-VIS Spectrophotometer and monitoring the
formation of the glutathione (2.5 mM in assay) and 1-chloro-2,4-dinitro-
benzene (1 mM in assay) conjugate at 340 nm (ε = 9.6 mM$^{-1}$ cm$^{-1}$) in
0.1 M potassium phosphate buffer, pH 6.5, at 25 °C according to Habig
et al. (22).

Reaction of 17β-IES with Glutathione S-transferase, Isozyme 1-1—
Glutathione S-transferase (0.2 mg/ml, 7.8 µM enzyme subunits) was
incubated with 0.1 M potassium phosphate buffer, pH 7.0, at 37 °C with
various concentrations of 17β-IES. Control enzyme samples were incubated
under the same conditions but without 17β-IES. At various time
points, an aliquot was removed from the incubation mixture, diluted,
and assayed (30 µl) for residual activity.

Measurement of Incorporation of 17β-IES into Glutathione S-Trans-
ferase—Glutathione S-transferase (0.2 mg/ml) was incubated with 500
µM [14C]17β-IES at pH 7.0 under standard reaction conditions. Aliquots
were withdrawn at various times, and excess reagent was removed by
the gel centrifugation method using two successive Sephadex G-50
columns (5 ml) equilibrated with 0.1 M potassium phosphate buffer, pH
7.5 (23). The protein concentration in the filtrate was determined using
the Bio-Rad protein assay, based on the Bradford method, using a
Bio-Rad 2500 RIA plate reader with a 600-nm filter (24). Unmodified
GST 1-1 was used to generate the standard concentration curve. The
amount of reagent present was determined by radioactivity using a
Packard 1500 Liquid scintillation counter. Incorporation was expressed
as mol 17β-IES/mol of enzyme subunit.

Preparation and Separation of Proteolytic Digest of Modified Gluta-
thane S-Transferase—Glutathione S-transferase (0.2 mg/ml) was
incubated with 500 µM [14C]-labeled 17β-IES at pH 7.0 under standard
reaction conditions for 3 h, at which time the enzyme was maximally
inactivated. Excess reagent was removed as described above. Solid
guanidine HCl was added to make a 5 M guanidine-HCl solution and
was incubated for 1 h at 37 °C to denature the protein, followed by
treatment with 10 mM N-ethylmaleimide at 25 °C for 30 min to block
free cysteine residues. The solution was then dialyzed against 6 liters of
10 mM ammonium bicarbonate, pH 8, at 4 °C with one change for a total
of 18 h, after which the sample was lyophilized.

The enzyme was solubilized by adding 250 µl of 8 M urea in 10 mM
ammonium bicarbonate, pH 8.0, and incubating at 37 °C for 1 h. The
solution was then diluted with 10 mM ammonium bicarbonate to bring
the final concentration of urea to 2 M. Chymotrypsin was added (10%
(w/w) at 2 h intervals while incubating at 37 °C. The ester bond between
the iodoacetic acid and estradiol-3-sulfate was subsequently hydrolyzed
b $\times$ NaOH to yield 0.2 N NaOH and then incubating the enzyme
digest at 25 °C for 2 h. The solution was then neutralized by adding HCl
to yield 0.2 N. The solution was filtered through a 0.45 µm filter, with no loss of radioactivity and was subjected to HPLC.

The chymotryptic peptides were fractionated by a Varian 5000 LC
equipped with a Vydac C$_{18}$ reverse-phase column equilibrated with
Solvent A (0.1% trifluoroacetic acid in water). At a flow rate of 1 ml/min,
the peptides were separated by a linear gradient from 0% to 20%
Solvent B (0.1% trifluoroacetic acid in acetonitrile) in 100 min followed
by a linear gradient to 100% Solvent B in 30 min. The eluate was monitored by A$_{220}$ and 1-ml fractions were collected. An aliquot (300 µl)
from each fraction was added to 5 ml of Liquiscent to test for
radioactivity.

Sequence Determination of Separated Peptides—The amino acid se-
quencies of purified peptides were determined on an Applied Biosystems
model 470A gas phase protein/peptide sequencer, equipped with a
model 120A phenylthiohydantoin analyzer.

Molecular Modeling—Molecular modeling was conducted using the
Insight II modeling package from Molecular Simulations, Inc. on an
Indigo 2 work station from Silicon Graphics. The model of rat GST 1-1

}\end{enumerate}
The reaction obeys pseudo-first order kinetics with a rate constant of 

\[ k_{obs} = k_{max}/(1 + (K_I/17\beta-IES)) \]

A least squares fit of the data yields 

\[ K_I = 71.4 \mu M \]

and 

\[ k_{max} = 0.0133 \text{ min}^{-1} \]

was constructed as described previously (19) based on the known crystal structure of human liver isozyme 1-1 (1GUH). The structure of 17\beta-IES was constructed using the Builder module. Docking of 17\beta-IES was done manually based on the energy minimized structure of 17\beta-estradiol-3,17-disulfate docked into isozyme 1-1 (15).

**RESULTS**

**Inactivation of Rat Liver Glutathione S-transferase 1-1 by 17\beta-IES**—Incubation of rat GST 1-1 (0.2 mg/ml, 7.8 \mu M enzyme subunits), with 300 \mu M 17\beta-IES, when assayed with 1-chloro-2,4-dinitrobenzene and glutathione, as described under “Experimental Procedures.” A, semilog plot of enzyme activity at time \( t \) (\( E_0 \)) versus time, \( B \), semilogarithmic plot of \( [E_0 - E_t]/[E_0 - E_i] \) versus time, where \( E_0 \) is the original enzyme activity, and \( E_i \) is the enzyme activity at long times, which is 0.6 (\( E_0 \)). The apparent rate constant (\( k_{obs} \)) determined from this graph was 0.0125 min\(^{-1}\).

**Concentration Dependence of the Rate of Inactivation—GST 1-1 (0.2 mg/ml, 7.8 \mu M enzyme subunits)** was incubated with 20–300 \mu M of 17\beta-IES as described above, to determine the rate of inactivation at various reagent concentrations (Fig. 3). The apparent rate constant \( k_{obs} \) exhibits a nonlinear dependence on reagent concentration. This type of curve is typical of an affinity label, suggesting that a reversible enzyme-reagent complex is formed prior to the irreversible modification of the enzyme (26). The curve can be described by the equation \( k_{obs} = k_{max}/(1 + (K_I/17\beta-IES)) \), where \( K_I \) is the apparent dissociation constant of the enzyme-reagent complex, and \( k_{max} \) is the maximum rate of inactivation at saturating concentrations of the reagent. A least squares fit of the observed data yields 

\[ K_I = 71.4 \mu M \]

and 

\[ k_{max} = 0.0133 \text{ min}^{-1} \]

**Effect of Ligands on the Inactivation Rate of GST 1-1 by 17\beta-IES**—Various ligand analogues were added to the reaction mixture to determine whether they could protect against the inactivation of the enzyme by 100 \mu M 17\beta-IES. The results, given in Table I, are expressed as \( k_{+L}/k_{-L} \), where \( k_{+L} \) is the rate constant for inactivation in the presence of a particular ligand, and \( k_{-L} \) is the rate constant for inactivation in the absence of a particular ligand. Glutathione derivatives (Table I, lines 2 and 3) offer some protection, with the protective effect increasing with an increase in alkyl chain length. The 5 mM mixture to determine whether they could protect against the inactivation of the enzyme by 100 \mu M 17\beta-IES. The points are experimental and the line is the theoretical fit to \( k_{obs} = k_{max}/(1 + (K_I/17\beta-IES)) \). A least squares fit of the data yields 

\[ K_I = 71.4 \mu M \]

and 

\[ k_{max} = 0.0133 \text{ min}^{-1} \]

**TABLE I**

Effects of enzyme ligands on the inactivation of glutathione S-transferase by 300 \mu M 17\beta-IES

| Ligand added | \( k_{+L}/k_{-L} \) |
|--------------|-----------------|
| 1. None      | 1.00            |
| 2. S-Methyl glutathione (5 mM) | 0.74 |
| 3. S-Hexyl glutathione (5 mM) | 0.37 |
| 4. \( \Delta^2 \)-Androstene-3,17-dione (500 \mu M) | 1.00 |
| 5. 17\beta-Estradiol-3-sulfate (100 \mu M) | 0.40 |
| 6. 17\beta-Estradiol-3-sulfate (500 \mu M) | 0.25 |
| 7. 17\beta-Estradiol-17-sulfate (100 \mu M) | 0.45 |
| 8. 17\beta-Estradiol-17-sulfate (500 \mu M) | 0.14 |
| 9. 17\beta-Estradiol-3,17-disulfate (100 \mu M) | 0.50 |
| 10. 17\beta-Estradiol-3,17-disulfate (500 \mu M) | 0.00 |

\( k_{+L}/k_{-L} \) was determined by the ratio of initial inactivation rate with ligand present to that observed in the absence of ligand.

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**FIG. 2.** Inactivation of glutathione S-transferase, isozyme 1-1, by 17\beta-IES. A solution of 0.2 mg/ml GST 1-1 was incubated with 300 \mu M 17\beta-IES in 0.1 \text{ M} potassium phosphate buffer, pH 6.5, at 37 °C. Activity was measured using the substrates 1-chloro-2,4-dinitrobenzene and glutathione, as described under “Experimental Procedures.” 

**FIG. 3.** Concentration dependence of \( k_{obs} \) for the inactivation of glutathione S-transferase by 17\beta-IES. GST (0.2 mg/ml) was incubated with a range of concentrations of 17\beta-IES under the same conditions as Fig. 2. At each concentration \( k_{obs} \) was calculated as illustrated in Fig. 2B, with \( E_0 = 0.6 (E_0) \). The points are experimental and the line is the theoretical fit to \( k_{obs} = k_{max}/(1 + (K_I/17\beta-IES)) \). A least squares fit of the data yields 

\[ K_I = 71.4 \mu M \]

and 

\[ k_{max} = 0.0133 \text{ min}^{-1} \]
site but distinct from it. Electrophilic substrates, such as 3\(^{17}\)-androstene-3,17-dione (Table I, line 4), do not provide any protection. In contrast, including steroid sulfates, such as 17\(^{17}\)-estradiol-3,17-disulfate, cause a striking decrease in the observed inactivation rate constant (lines 5–10). Because these steroid sulfates are known to bind at a nonsubstrate steroid site (15), the results indicate that 17\(^{17}\)-IES is reacting within this nonsubstrate steroid binding site.

Incorporation of Radioactive 17\(^{17}\)-IES into GST 1-1—GST 1-1 (0.2 mg/ml) was incubated with 300 \(\mu\)M \(^{14}\)C-17\(^{17}\)-IES. A time-dependent incorporation of \(^{14}\)C-17\(^{17}\)-IES was observed concomitant with the decrease in enzyme activity. A plot of the percentage of maximum inactivation versus net incorporation (Fig. 4) extrapolates to about 0.46 mol of \(^{14}\)C-labeled reagent/mol enzyme subunit.

Isolation and Characterization of Chymotryptic Peptides from 17\(^{17}\)-IES Modified GST 1-1—Maximally inactivated GST 1-1 was prepared and digested with chymotrypsin. The digest was fractionated by HPLC using a reverse-phase column (C\(_{18}\)) equilibrated with 0.1% trifluoroacetic acid and an acetonitrile gradient (Fig. 5). One radioactive peptide peak was observed on HPLC. Because the ester linkage of 17\(^{17}\)-IES (Fig. 1) was hydrolyzed before the digest was applied to HPLC, the steroid moiety was removed, and the peptide is expected to be labeled with the radioactive carboxymethyl group. The fractions corresponding to this peak were pooled, lyophilized, and subjected to gas phase amino acid sequencing. The results are shown in Table II. The sequence Glu-Xaa-Ile-Arg-Trp corresponds to residues 16–20 in the known amino acid sequence. None of the common phenylthiohydantoin derivatives was detected in cycle 2; instead, there was a peak with a retention time between that of phenylthiohydantoin-Ser and phenylthiohydantoin-Asn. This peak corresponds to that of a phenylthiohydantoin-carboxymethylcysteine standard, indicating that a Cys in this

**Table II**

| Cycle | Amino acid |
|-------|-----------|
| 1     | Glu (141) |
| 2     | Xaa\(^a\) |
| 3     | Ile (37)  |
| 4     | Arg (34)  |
| 5     | Trp (14)  |

\(^a\) Retention time is 7.8 min between PTH-Asn and PTH-Ser.
position had been modified. Thus, Cys$^{17}$ of GST1-1 is the amino acid target of 17β-IES.

**DISCUSSION**

17β-Iodoacetoxy-estradiol-3-sulfate acts as an affinity label of rat liver glutathione S-transferase isozyme 1-1. Upon incubation of the enzyme with 17β-IES, a time-dependent loss of activity is observed, yielding a maximum loss of 40% of the original activity. The rate of inactivation exhibits nonlinear dependence on reagent concentration, as is typical of an affinity label, for which an enzyme-reagent complex forms prior to irreversible modification. Partial protection against inactivation is provided by glutathione derivatives; long chain derivatives, such as S-hexylglutathione, provide more protection than do shorter chain derivatives, like S-methylglutathione, indicating that 17β-IES is binding in a site close to the glutathione site but not within the site. Electrophile substrate analogues, such as Δ$^5$-androstene-3,17-dione, do not offer any protection, demonstrating that 17β-IES does not bind within the electrophilic substrate site. Steroid sulfates are most effective in protecting against inactivation of GST, 1-1, with 17β-estradiol-3,17-disulfate providing complete protection. These results indicate that 17β-IES is binding and reacting within the nonsubstrate steroid binding site.

Upon maximum inactivation, about 0.5 mol of reagent is incorporated per mol enzyme subunit or 1 mol of 17β-IES/ enzyme dimer, and Cys$^{17}$ is the only amino acid that is modified. In previous work, based on the crystal structure of glutathione S-transferase from the parasitic worm *Schistosoma japonica* in complex with praziquantel, an anti-schistosomal drug bound in the cleft between the subunits, only 1 mol of praziquantel is bound per mol of enzyme dimer (27). Photoaffinity labeling of rat liver GST 1-1 by glutathionyl S-[4-(succinimidyl)-benzophenone] also results in one subunit being modified (28). Other precedence for binding only 1 mol reagent/mol enzyme dimer comes from work with large conjugation products, such as S-[(2,2,5,5-tetramethyl-1-oxy-3-pyrrolidinyl)-carbamoyl]methyl]glutathione (29), and the aflatoxin glutathione conjugate, 8,9-dihydro-8-(S-glutathionyl)-9-hydroxyl-aflatoxin, which bind to a class glutathione S-transferases with a stoichiometry of 1 mol/mol dimer (25).

In the case of glutathionyl S-[4-(succinimidyl)-benzophenone], only one subunit is modified, yet the enzyme is completely inactivated. The modification of one subunit thus can abolish the enzyme activity of both subunits and, because this label does not occupy the nonsubstrate site, the inhibition is probably the result of a subtle conformational change rather than a physical barrier to the binding of the substrate (28). There is also complete inactivation by the aflatoxin conjugate, although in this case, the bound conjugate extends into the cleft and therefore may be inhibiting completely either because it is blocking access to the active site of the unmodified subunit or because it induces a conformational change (25).

In the present case, maximum reaction with 17β-IES results in the loss of only 40% of activity; it is likely that the unmodified subunit retains full activity, whereas the other subunit with modified Cys$^{17}$ is 80% inactive. Incorporation of 17β-IES on one subunit apparently prevents a second molecule from binding to and reacting with the other subunit, but, in contrast to the previous examples, this does not cause complete inactivation of both subunits. The 17β-IES reacts at the steroid site, which is distinct from the active site, and thus there is still some residual activity in the modified subunit, whereas the catalytic site on the other subunit functions independently and is completely active. These results indicate that the observation of apparent cooperativity between the subunits of glutathione S-transferase depends on the particular binding site that is being examined.

A homology model for the rat 1-1 isozyme was generated from the crystal structure of the human glutathione S-transferase 1-1. The reagent was manually docked into the model based on an energy-minimized structure of 17β-estradiol-3,17-disulfate bound to GST 1-1 and the assumptions that the iodoacetoxy group of the 17β-IES must be close to the sulphydryl group of Cys$^{17}$ as well as in an orientation to modify only one subunit. The structure shown in Fig. 6 meets these requirements.

In the proposed model, there is 1 mol of 17β-IES bound in the cleft between the subunits of the enzyme. The reactive iodoacetoxy group is about 3.4 Å from the sulphydryl group of Cys$^{17}$ and the sulfate group is about 3.1 Å from the guanidino group of Arg$^{14}$.

**Fig. 6. Homology model of rat GST 1-1 complexed with 17β-IES, constructed as described under “Experimental Procedures.”** The reactive iodoacetoxy group is about 3.4 Å from the sulphydryl group of Cys$^{17}$, and the sulfate group is about 3.1 Å from the guanidino group of Arg$^{14}$.

In summary, 17β-IES functions as an affinity label of the nonsubstrate steroid site of rat liver glutathione S-transferase, isozyme 1-1. Upon incubation with 17β-IES, the enzyme loses 40% of its activity, incorporates about 0.5 mol of reagent/enzyme subunit, and is modified only at Cys$^{17}$. Protection against inactivation by 17β-IES is best provided by steroid sulfates, such as 17β-estradiol-3,17-disulfate, indicating that Cys$^{17}$ is within the nonsubstrate steroid binding site of the enzyme and that its binding is more specific than that of 3β-IDA because of the interaction of the sulfate group with the side chain of Arg$^{14}$. Based on analysis of molecular models, this nonsubstrate site is located within the cleft between the two subunits of the enzyme.

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