Human placenta glutathione transferase (EC 2.5.1.18) undergoes an oxidative inactivation which leads to the formation of an inactive enzymatic form which is homogeneous in several chromatographic and electrophoretic conditions. This process is pH dependent, and it occurs at appreciable rate in alkaline conditions and in the presence of metal ions. Dithiothreitol treatment completely restores the active form. -SH titration data and electrophoretic studies performed both on the oxidized and reduced forms indicate that one intrachain disulfide is formed, probably between the two faster reacting cysteinyl groups of each subunit. By the use of a specific fluorescent thiol reagent the disulfide forming cysteines have been identified as the 47th and 101th residues. The disulfide formation causes changes in the tertiary structure of this transferase as appears by CD, UV, and fluorometric analyses; evidences are provided that one or both tryptophanyl residues of each subunit together with a number of tyrosyl residues are exposed to a more hydrophilic environment in the oxidized form. Moreover, electrophoretic data indicate that the subunit of the oxidized enzyme has an apparent molecular mass lower than that of the reduced transferase, thereby confirming structural differences between these forms.

Glutathione transferase (EC 2.5.1.18) (GST) is a family of enzymes found in numerous species and in many tissues of mammals (1, 2). They represent one of the most efficient biological systems for the detoxification of electrophilic alkylation agents (3). They may also be implicated in other cellular metabolisms such as binding of hydrophobic compounds, i.e. drugs and bilirubin, steroid isomerization, and reduction of hydroperoxides (3).

The mammalian enzymes have been grouped into three distinct classes named Alpha, Mu, and Pi on the basis of several criteria including amino acid sequence, substrate, and inhibitor specificity and immunological properties (4). All the cytosolic enzymes have a dimeric structure due to a noncovalent association of identical or different subunits with a molecular mass of 23-28 kDa (3). Moreover, each monomer contains one binding site for glutathione (GSH) (G-subsite) and another for the hydrophobic substrate (H-subsite) (3).

Several studies have been performed to define the catalytic mechanism of this enzyme, but this remains obscure at the present; similarly, the topography of the subsites is not clarified despite the primary structure of a number of isoenzymes established so far (3). At this regard one important question is the role, if any, of the sulfhydryls present in each subunit of the Pi class isoenzymes. It has been recently observed that the covalent modification of a single cysteine residue/monomer causes a dramatic loss of activity of the homodimeric Pi isoenzymes such as the rat GST 7-7 (5), the human placenta GST-π (5, 6), the mouse GST M II (5), and the horse erythrocyte GST (7). This sulfhydryl is the most reactive among the 4 cysteine residues of each subunit, and it has been identified as the 47th amino acid both in the rat 7-7 and human placenta π isoenzymes (5, 6). Whether this thiol group is important for the maintenance of a catalytically active structure or whether it is implicated in the catalytic mechanism remains to be clarified. Probably related to the integrity of this residue are also the observations that oxidizing agents such as H₂O₂ or disulfides cause a loss of the GST activity (4, 5, 7-10) and that a number of GST purifications were performed in the presence of reducing agents to prevent enzyme inactivation. These data suggest a correlation between the redox state of the Pi class GST and its activity; this was recently suggested by Shaffer et al. (8) who observed that the bovine placenta GST exists in an active reduced form and a less active oxidized form. These forms behave differently in sedimentation analysis, gel chromatography, and gel electrophoresis (8). Unfortunately, they did not perform further characterizations of the oxidized form. Therefore, up to now, the term "oxidized GST" is ambiguous since the number of involved sulfhydryls and their identification in the primary structure are not yet defined, the homogeneity of the oxidized form(s) as well as the factors affecting this process are unknown. The aim of the present paper is a quantitative approach for the characterization of the redox states of the human placenta GST.

We present evidence that one intrachain disulfide bond may be formed in each subunit of this transferase between cysteines 47 and 101. This occurs in metal-catalyzed and pH-dependent processes. This oxidative reaction yields one single inactivating enzyme population which appears under several chromatographic and electrophoretic analyses. Moreover, these techniques together with UV, circular dichroism, and fluorescence data let us hypothesize remarkable
conformational differences between the reduced and oxidized forms.

**EXPERIMENTAL PROCEDURES**

**Enzyme Preparation**—Human placenta GST \( \alpha \) was prepared as previously described (11). About 20 mg of this enzyme were further purified by CM25 column (2 x 10 cm) equilibrated with 10 mM K-phosphate buffer, pH 6.8 containing 1 mM EDTA. The acidic isoform was not retained whereas several protein contaminants were bound to the resin. Aliquots of GST were then treated with 10 mM dithiothreitol (DTT) at pH 8.0 (0.1 M K-phosphate buffer and 1 mM EDTA) for 60 min at 37 °C to achieve the maximal specific activity. DTT was removed by a G-25 Sephadex column (1 x 40 cm) equilibrated with 0.1 M K-phosphate buffer, pH 7.0 (or other buffers depending by the subsequent experimental conditions) and 1 mM EDTA. This activity value remains unchanged for at least 3 h at 37 °C (at pH 7.0) after the Sephadex chromatography. The active isoform appears homogeneous on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), disc-gel electrophoresis under non-denaturing conditions and on fast protein liquid chromatography (FPLC). This GST is named in this paper as "fully reduced GST" (220 units/mg). By incubating such enzyme in 0.1 M K-phosphate buffer, pH 8.0, for 48 h at 25 °C, we obtained an inactive form (<1 unit/mg) which is defined as "fully oxidized GST" or "fully inactivated GST." The inactive form obtained after reaction with stoichiometric or catalytic amounts of cupric ions was dialyzed overnight against the same buffer. To verify any experiment.

**Enzyme Activity**—GST activity was assayed spectrophotometrically at 340 nm (12). Standard incubation mixture contained 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) and 2 mM GSH in 2 ml (final volume) of 0.1 M K-phosphate buffer, pH 7.0, containing 1 mM EDTA. One enzyme unit is the amount of GST that catalyzes the conjugation of 1 n mole of GSH/min at 37 °C. Spectrophotometric measurements were corrected for the spontaneous reaction of GSH with CDNB. Protein concentration was determined as described by Lowry (13). Molarity of GST was calculated on the basis of a molecular mass of 46 kDa.

**Inactivation Experiments**—About 0.7 mg of fully reduced GST (15 \( \mu \)m) were incubated at 25 °C in the presence of 0.1 M K-phosphate buffers, pH 7.0-8.0, and 0.1 M Tris-HCl buffers, pH 8.5-9.0, in the presence or absence of 1 mM EDTA (1 ml final volume). At various times 10-\( \mu \)l aliquots were assayed for GST activity as above described.

**Thiol Group Titration**—Protein sulfhydryls were titrated essentially by following the Ellman and Grassetti and Murray procedures (14, 15); about 230 \( \mu \)g of oxidized or reduced GST were reacted with

**Oxidative Inactivation of Placenta GST-\( \alpha \)—GST spontaneously inactivates in a pH-dependent process (Fig. 1, Table I). The inactivation is faster by increasing the pH values and it follows pseudo-first order kinetics (Fig. 1B). In our experimental conditions the pH-independent inactivation due to a
Redox Forms of GST

TABLE I

Standard inactivation procedures at various pH values and activity determinations were performed as described under "Experimental Procedures."

<table>
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<tr>
<th>pH</th>
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<th>min⁻¹</th>
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</tr>
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<tr>
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<td>+</td>
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<td></td>
</tr>
<tr>
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<td>25 °C</td>
<td>-</td>
<td>0.0013</td>
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</tr>
<tr>
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<td>25 °C</td>
<td>+</td>
<td>0.0000</td>
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</tr>
<tr>
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<td>25 °C</td>
<td>-</td>
<td>0.0000</td>
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</tr>
<tr>
<td>7.0</td>
<td>25 °C</td>
<td>+</td>
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</tbody>
</table>

TABLE II

Rate constants for dithiothreitol and glutathione reactivation of the fully inactivated GST

The reactivations were carried out in 0.07 M potassium phosphate buffers, pH 7.0 and 8.0, and Tris-HCl buffer, pH 9.0, with 4 mM DTT or 4 mM GSH at 37 °C. Fully inactivated GST was 4.6 μM. At fixed times 5-μl aliquots were assayed for activity as described under "Experimental Procedures."

<table>
<thead>
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<th>pH</th>
<th>Pseudo-first order constants</th>
<th>min⁻¹</th>
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<tr>
<td>DTT</td>
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</tr>
<tr>
<td>GSH</td>
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</tr>
<tr>
<td>GSH</td>
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<tr>
<td>GSH</td>
<td>9.0</td>
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</table>

solvation of this transferase and occurring at low enzyme concentration (19) is negligible as appears from the experiment performed at pH 7.0. The inactivated enzyme at pH 9.0 after 8 h of incubation and that obtained after 24 h of incubation at pH 8.0 have approximately the same activity of less of 1%. The time course of this process is affected by the temperature (Table I) and does not occur under nitrogen (data not shown). The presence of metal-chelating agents such as EDTA dramatically lowers the inactivation rate (Fig. 1, Table I) thereby indicating a probable involvement of metal ions as catalysts. As shown in Fig. 1 the presence of a stoichiometric amount of cupric ions enhances about 10-fold the inactivation rate of GST. Catalytic amounts of cupric ions also yield a higher rate of inactivation, but in that case a more complex kinetic behavior has been observed. Both the spontaneous and copper-catalyzed inactivated GSTs recovered completely the original activity by treatment with 4 mM DTT within 10 min of incubation at pH 8.0 (37 °C). The reactivation process is pH dependent, and it follows a pseudo-first order kinetic (Table II). GSH yields much slower reaction rates than DTT at all pHs tested; this could reflect both a difference in the redox potential values of the oxidized GST and glutathione or a steric hindrance for GSH in its reaction with the oxidized protein groups.

All these data point out the probable involvement of a number of protein cysteine residues in a reversible redox process as also hypothesized for the bovine placenta GST (8) which undergoes a similar reversible inactivation in the presence of H₂O₂.

Thiol Group Titration—Human placenta GST has 4 cysteine residues/subunit. These data arise from the complete primary structure as deduced from the correspondent cDNA and the gene structure (20, 21). Direct titration of sulhydryl groups in the placenta GST can be performed with thiol-specific reagents such as DTNB or DTDTP although it has been observed that the class Pi isoenzymes possess a number of masked –SH groups that do not react with these reagents even under denaturing conditions (7). In our experimental conditions the fully active enzyme has two fast reacting sulhydryls/subunit both titrable with DTNB and DTDTP and a third slow reactive thiol group (Fig. 2, Table III). The fourth cysteine residue is not titrable in 8 M urea, 6 M guanidine, or after NaBH₄ treatment and urea denaturation (Table III). Upon the contrary, the fully inactivated GST (both spontaneously and copper catalyzed) has only one slow reactive –SH group/subunit (Fig. 2, Table III). From these data it appears that the fully inactive GST differs from the active form of two disulfides/dimer, but do not clarify whether these are intrachain or interchain bridges. On the other hand these disulfides seem to involve only the four faster reacting sulhydryls.

Other interesting data arise from the –SH group titration with DTNB performed on the native enzyme at low pH values. As shown in Fig. 2 at pH 7.0 and 5.0 only four –SH groups are detectable. At pH 5.0 two fast and two slow reacting...
sulfhydrils can be observed. A more detailed kinetic analysis performed accordingly to Frost and Pearson (22) (data not shown) allows the calculation of apparent pseudo-first order constants of 0.34 and 0.031 min⁻¹, respectively. During the experiment performed at pH 5.0, we also evaluated the loss of activity as a function of the titrated sulfhydrils. The enzymatic activity was reduced to 10% when two sulfhydrils are reacted with DTNB thereby confirming the importance of only 1 reactive cysteine residue/subunit as previously observed with other thiol reagents (6, 7). The observed spontaneous disulfide formation in GST let us explore the possibility that this may also be formed during the reaction with DTNB, for example, by a thiol-disulfide exchange between a protein -SH group and the protein-DTNB mixed disulfide. When the DTNB-reacted enzyme at pH 7.0 (four titrated -SH groups) was purified by a G-25 chromatography and treated with 1 mM DTT, the retrotitration data reported in Fig. 3 clearly indicate that four TNB⁺ are bound to the enzyme, and therefore no protein disulfide bridge has been formed.

Electrophoresis under Denaturing and Nondenaturing Conditions—SDS-PAGE was performed both on the spontaneous and copper-inactivated GSTs and compared with the fully active enzyme. As reported under "Experimental Procedures," the standardized procedure by Laemmli (16) was modified by omitting Et-SH in the sample and in the run buffer. Under these conditions the active enzyme gives a single band with an apparent molecular mass of 23 kDa identical to that found in the standardized conditions with Et-SH (11) (Fig. 4). On the other hand both the inactivated forms appear as homogeneous components with the smaller apparent molecular mass of 20.5 kDa. Pretreatment of the inactivated GST with 1 mM DTT prior electrophoresis restores the single band at 23 kDa. The lack of bands with molecular mass higher than 23 kDa points out that no intersubunit disulfide exists in these inactivated forms. Moreover, these results suggest that the spontaneous and copper-catalyzed oxidations lead to the formation of similar homogeneous populations of inactive transferease with subunits of smaller apparent molecular mass; this may be due to a conformational change of the molecular shape as also hypothesized for the oxidized forms of the bovine

Fig. 3. Retrotitration of the DTNB-treated GST. Fully reduced GST (1.5 µM) were reacted with DTNB (1 mM) in 2 ml (final volume) of 0.1 M K-phosphate buffer, pH 7.0. The absorbance at 412 nm reached a plateau in about 40 min corresponding to four -SH groups titrated/mol of GST. The enzyme was almost inactivated. The excess of DTNB was removed by a Sephadex G-25 column (1 x 40 cm) equilibrated with 0.1 M K-phosphate buffer, pH 8.0. The enzyme was concentrated to 2.5 ml and reacted with 1 mM DTT. The increase of absorbance at 412 nm was followed in continuous and at fixed times 10-µl aliquots were tested for GST activity. ○, TNB⁺ released/mol of GST; □, percentage of GST activity.

isoenzyme (8). We also observed a different chromatographic behavior with identical amounts of oxidized and reduced GSTs were stained with Coomassie Brilliant Blue R-250 on gel. These differences were also detected in solution by following the Bradford procedure (23); the oxidized form gives an absorption at 595 nm about 20% lower than that of the active form. This is a further indication of structural differences between these forms. The homogeneity of the GST-oxidized forms and their likeness were also supported by the disc gel electrophoresis under nondenaturating conditions (Fig. 4). Additionally in this case, the oxidized forms migrate as a single band with a higher mobility than the reduced GST. Under these conditions, however, we cannot establish whether the higher mobility of the inactive GSTs is due to a structural modification or to an enhancement of the net negative charge on the protein. Moreover, both the oxidized GSTs are indistinguishable on chromatofocusing analysis giving a single peak with a retention time (24 min) different from that of the active enzyme (30 min). This result is again an indication that both the spontaneously and copper-inactivated GSTs are identical and that the disulfide formation causes a change of the chemico-physical properties of this transferease.

Identification of the 2 Cysteinyls Involved in the Disulfide Bridge—The spontaneously oxidized transferease was denatured in 6 M guanidinium chloride and then reacted with monoiodoacetate to alkylate other sulfhydrils not involved in the disulfide bond as described under "Experimental Procedures." The modified enzyme was then digested with TPCK-trypsin for 24 h. After this procedure the peptide mixture was reduced with 1 mM DTT and reacted with 50 mM ANM, a specific fluorescent probe for -SH-containing compounds recently used for the detection of the high reactive cysteine 47 of this isoenzyme (6). The HPLC pattern of this peptide mixture revealed only two major fluorescent peaks eluted after 29 and 33 min, both exhibiting approximately the same fluorescent intensity (Fig. 5). HPLC of the peptide mixture reacted with ANM without any DTT treatment lacks of these major fluorescent peaks, thereby indicating the absence of any free sulfhydrils in the peptides. The automated Edman degradation gave the following amino acid sequence for the first faster fluorescent peak: Asp-Gln-Gln-Glu-Ala-Ala-Leu-Val-Asp-Met-Val-Asn-Asp-Gly-Val-Glu-Asp-Leu-Arg-Xxx-Lys; the same analysis performed on the second fluorescent
peak gave the following sequence; Ala-Ser-Xxx-Leu-Tyr-Gly-Gln-Leu-Pro-Lys. By comparing these sequences with the complete primary structure of this isoenzyme (20, 21) the 2 modified cysteines can be identified as the 101 and 47 residues, respectively.

**CD Spectra**—All the spectroscopic analyses were performed on the spontaneously inactivated GST to avoid any interference due to cupric ions. Moreover, since the spontaneous oxidation occurs at a very low rate, we always obtained spectroscopic data by starting with the fully oxidized GST and by following the reactivation process by DTT or Et-SH. In particular, circular dichroism experiments have been performed with DTT as reducing agent since neither the reduced DTT nor the disulfide bond in the oxidized DTT give significant dichroism over the concentrations and the spectral range studied (24). From Fig. 6A, it is evident that CD spectrum of the fully active enzyme markedly differs from that of the oxidized form in the 250–300-nm region. The interpretation of such spectral changes cannot be made without an accurate knowledge of the contributions of tryptophans, tyrosins, and other chromophores to the total spectrum of the reduced form of placenta GST. These data are unknown up to now although the CD spectra of the Pi isoenzymes have been reported and compared with those of the Mu and Alpha classes isoenzymes (25). In spite of this lack several considerations can be made. First, the simple subtraction of the spectrum of the reactivated GST from that of the oxidized form generates a broad negative band with a maximum at 270 nm and a second smaller peak at about 283 nm (Fig. 6B), thereby indicating at least two groups of chromophores to be involved in this spectral change. A second observation is that change of the ellipticity values at 270 nm parallels the reactivation kinetic (Fig. 6B, inset). It has been claimed that low energy disulfide bond transitions may occur at any wavelength between 250–340 nm, depending on the environment of the disulfide and on the dihedral angle and that they can generate a conspicuous negative contribution to the ellipticity band (26–30). Except for a few proteins, generally the CD peaks of protein disulfides are broad with no clear-cut fine structure (31). It is interesting to note that the peak centered at 270 nm resembles that caused by the optically active disulfides of the oxidized forms of human somatotropin (24) and human apolipoprotein B (32). Tentatively we may assign the major band centered at 270 nm to the disulfide of the oxidized GST and the second band at 283 nm to aromatic residues involved in some structural change. On the contrary, the far UV–CD spectra of the oxidized and reduced forms of GST are very similar (data not shown) thereby indicating that no consistent differences in the secondary structure occur between these forms.

**UV Spectra**—For UV differential spectra between the reduced and oxidized forms of GST, several parameters have been optimized to obtain reliable spectrophotometric data. We followed the reduction process of oxidized GST at pH 8.0 and in the presence of 1 mM Et-SH; under these conditions Et-SH which has a pKsH = 9.6 (33) is largely undissociated. The small amount of the ionized form (about 25 μM) is stoichiometric in respect to the oxidized protein (28 μM), however, the presence of an high amount of undissociated Et-SH as “buffer” makes constant the concentration of the dissociated form during the reduction process. These conditions are important since the thiolate ion absorbs at 230–240 nm and any change of its concentration yields interferences in the UV region even at higher wavelengths. Similarly the use of DTT as reducing agent was avoided since its oxidized form absorbs at 283 nm. We also tested that the spontaneous autoxidation of Et-SH, which leads to the formation of a disulfide absorbing at 250 nm, was negligible until the reactivation went to completion. As shown in Fig. 7, the reduction of GST is accompanied by UV perturbations in the 270–310-nm region. It is well known that the 270–310-nm perturbations are mainly due to changes in the environment of tryptophan and tyrosyl residues (34). The reduced form of GST shows an increased absorption in this region with maxima centered at 291, 287, 285, 280, and 274 nm. A maximum perturbation with Δε291 = 710 M−1 cm−1 was obtained after 90 min of incubation. Moreover, a broad peak can be observed near 300 nm. On the basis of the reported absorption difference for tyrosyls and tryptophans due to several perturbants (34), the peaks at 291, 285, and 274 nm may be due to an enhanced hydrophobicity near one or both tryptophans of GST.
Fig. 7. Differential UV spectra. Fully oxidized GST (28 μM) were incubated with 1 mM Et-SH in 2.5 ml of 0.1 M K-phosphate buffer, pH 8.0 (25 °C). BK cuvette contained all reagents except Et-SH. At fixed times, 10-μl aliquots were assayed for GST activity. Dotted line, differential UV spectrum at zero time; solid line, differential UV spectrum after 90 min of reactivation. Inset, kinetics of the UV perturbations at 291 nm (C) and 300 nm (Δ). UV data are reported as percentage of the maximal UV perturbations (100 min of reactivation).

Fig. 8. Fluorescence analysis. Fully oxidized GST (28 μM) were incubated with 1 mM Et-SH in 2.5 ml of 0.1 M K-phosphate buffer, pH 8.0 (22 °C). Sample was excited at 280 nm. At fixed times 10-μl aliquots were assayed for GST activity. A, fluorescence emission spectra (λex = 280 nm) at zero time (a), after 40 min of incubation (b), and after 100 min of incubation (c). B, kinetics of the fluorescence quenching (●) and blue shift (○) compared with the reactivation kinetic (□). Data are expressed as percentage of the maximal perturbations (110 min of reactivation).

Our experimental data indicate that the human placenta GST π may undergo an oxidative inactivation due to an intrachain disulfide formation. This process occurs at appreciable rate only in alkaline conditions and probably requires traces of metal ions as demonstrated by the inhibiting effect of EDTA (Fig. 1). This is also confirmed by the enhancement of the oxidation rate produced by catalytic amounts of cupric ions. Both the “spontaneous” and the copper-catalyzed processes lead to the formation of an inactive transferase which is homogeneous on SDS-PAGE, FFLC, and PAGE under non-denaturing conditions (Fig. 4). The analytical data on SDS-PAGE, performed without reducing agents, clearly indicate that no interchain disulfide bonds have been formed. Moreover, the subunit of the oxidized form of GST shows an apparent molecular mass of 20.5 kDa which is smaller than the corresponding reduced form. Since a complete recovery of the original band of 23 kDa has been observed after DTT treatment (Fig. 4), a conformational modification may be supposed due to a disulfide formation. This was then confirmed by the spectroscopic data which will be discussed below. Our data are indicative that the oxidation involves 2 cysteines among the 4 present in each subunit. These data arise from titration experiments performed with DTNB and DTDP (Table III and Fig. 2). In particular, with both these reagents it appears that among the three titrable sulhydryls/monomer, the 2 faster reacting cysteines are probably involved in the disulfide formation. The occurrence of a disulfide was confirmed by tryptic digestion of the oxidized transferase after blockage of the remaining free sulphydryls with monoiodoacetate. When the peptides were reduced by DTT and labeled with ANM, only two major fluorescent peaks were detected on HPLC (Fig. 5). This is an indication that only two sulphydryls have been oxidized in each subunit and that the disulfide is identical in both subunits.

The AA sequence of these peptides allowed the identification of the cysteine 47 and the cysteine 101 as involved in this disulfide. This may be informative about the tridimensional structure of the subunit of this isoenzyme: these cysteinyls being far in the primary sequence, it seems to be reasonable that in the folded native structure they must be sufficiently close to interact and then to form the disulfide bridge. The redox process is accompanied by changes of the tertiary structure of this protein as it appears by several spectroscopic evidences obtained during the reduction of the fully oxidized GST. For example, the differential CD spectrum between the reduced and oxidized forms (Fig. 6) shows a spectral modification centered at 283 nm probably caused by a structural change in some aromatic group. The second perturbation at about 270 nm has been tentatively explained as the contribution of the disulfide to the total spectrum of
the oxidized form. No differences have been observed in the far UV CD spectrum thereby indicating that no change in the secondary structure occurs. The perturbation of aromatic groups in the redox process of this transferase was confirmed by UV and fluorometric analyses. During the disulfide breakdown both the UV differential spectra in the 270–310 nm region (Fig. 7) and the blue shift of the intrinsic fluorescence (Fig. 8) are consistent with a movement of one or both tryptophanyla into a more hydrophobic environment. These experiments also indicate that a second kinetically different process occurs, probably due to the approaching of a negative charge near the tryptophan(s).

All these spectroscopic data allow hypothesis of a role for the crucial cysteine 47. It has been demonstrated that the inactivation is due to conformational modification by the steric hindrance of the –SH reagents is improbable as complete inactivation has been observed with many reagents of different sizes and charges (7). The involvement of this group in the catalytic mechanism of the GST Pi cannot be excluded, but there is no evidence for that at present. On the other hand the hypothesis for a structural role of this sulfhydryl is in agreement with the data in this paper: during the redox process involving the critical cysteine 47 both inactivation and structural change have been observed simultaneously. Therefore it should be important for the maintenance of a proper tertiary structure.

From another point of view these data put forth an interesting question about the possible regulation of the activity of this transferase in vivo, based on a redox process. It has been well known that many enzymatic activities may be modulated by redox reactions such as SH/SS exchange with biological disulfides (38–41). This has also been recently suggested for this isoenzyme (10). The present data open the new possibility that the activity of this isoenzyme may also be regulated by an intramolecular disulfide. We are now studying the possible existence of this inactive oxidized GST in several tissues and the possibility that this form is more easily attacked by proteolytic enzymes.

REFERENCES