

# Isolation, Characterization and Comparison of Antipeptide and Antiprotein Rabbit Antibodies to the $\pi$ -Isoform of Glutathione S-Transferase

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The main linear epitopes of  $\pi$ -glutathione transferase ( $\pi$ -GST, EC 2.5.1.18), an enzyme related to cancer progression in a restricted number of tumours, were identified by testing in ELISA the reactivities of polyclonal anti- $\pi$ -GST rabbit sera against a panel of 51 overlapping decapeptides, covering the whole 216-residue sequence of the protein. Several major reactivity peaks were detected, each covering two or three adjacent peptides. The most active fragments were then reconstructed by conventional solid-phase synthesis, linked to Sepharose, and used as affinity ligands for isolating specific anti- $\pi$ -GST antibody subsets.

A second group of antisera was then prepared in rabbits by using as immunogens some of the above described synthetic fragments, linked to a carrier protein, and antipeptide antibodies purified by affinity chromatography. An ELISA test was then performed, using as antigens a panel of peptides and different isoforms of GST, in order to establish whether antibodies isolated from total anti- $\pi$ -GST sera would display higher reactivity and specificity, as compared to traditional antipeptide antibodies. Binding data clearly confirm that the formers might be indeed better reagents for the detection and possibly quantitation of  $\pi$ -GST.

## Introduction

The glutathione transferases are a family of multifunctional proteins that act as enzymes in various detoxication processes, catalyzing the reaction of nucleophilic reduced glutathione with electrophilic compounds biotransformed from xenobiotics, including cancerogens (Jakoby, 1978; Chasseaud, 1979; Mannervik, 1985). Several molecular forms (isoenzymes) of GST are constitutively expressed by normal tissues, while few others, as rat GST-P and human  $\pi$ -GST, have attracted attention as reliable preneoplastic or neoplastic marker enzymes, the detection of which might facilitate analysis of carcinogenic processes (Seidegard *et al.*, 1986; Sato, 1989). Glutathione transferases in rat, human, mouse, as well as in several other species, have been extensively studied and partly characterized (for a review, see Tsuchida and Sato, 1992).

The major human isoenzymic cytosolic classes ( $\alpha$ ,  $\mu$ , and  $\pi$ ) share common structural properties (Mannervik *et al.*, 1985), and polyclonal antisera

to one isoform usually contain antibodies with unwanted crossreactivities with the others. Specific anti- $\pi$ -GST reagents might however be obtained by repeated absorptions of polyclonal sera or by generating monoclonal antibodies.

The most obvious approach for producing specific anti- $\pi$ -GST antibodies might be the immunization of rabbits with synthetic peptides selected from  $\pi$ -GST regions which are located on the surface of the protein and are not homologous in the other isoforms. The comparison of primary structures of glutathione transferases reveals that the isoforms do not apparently share high sequence homology, as revealed for instance by the different locations of the cysteine residues, by the length of the polypeptide chains, and by several amino acid insertions and deletions. Close sequence homology among isoforms occurs only at few amino acid stretches, suggesting that crossreactivity of anti-GST sera with all isoforms might be predominantly based on discontinuous conformational determinants.

Antibodies to peptides are however frequently not very specific, and crossreactivities with molecules sharing similar structural features are common. In addition, their reactivity with the intact

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protein may be low, even when the fragment used for immunization has been selected from surface domains of the macromolecule (Chersi *et al.*, 1997).

Alternatively, one might use immobilized synthetic peptides as affinity ligands for isolating, from polyclonal anti- $\pi$ -GST sera, antibody subsets directed to well-defined epitopes of the protein (Ullrich *et al.*, 1986; Chersi *et al.*, 1987). For this approach, one should preferably delineate in advance the major sequential epitopes of the protein, as by aid of an immunoenzymatic assay on a large panel of peptides prepared by the Multipin Synthesis method (Geysen *et al.*, 1985; Geysen *et al.*, 1986). Reactive peptides identified by this method can be then subsequently synthesised and used on a large scale affinity purification.

This second approach is obviously more complex, as it requires time and instrumentation for pins synthesis. On the other hand, it permits the isolation of antibodies that are presumably directed against epitopes in the native conformation. Those antibodies might thus differ from anti-peptide antibodies produced against the same fragment: for instance, their reactivity with the intact protein might be higher. As far as it concerns the specificity of antibodies for the  $\pi$ -isoform of GST, this feature is more difficult to predict (Chersi *et al.*, 1997), and should be experimentally determined.

As specificity and reactivity are prerequisites for a suitable utilization of antibodies, it might be of interest to state whether antibodies isolated from total anti- $\pi$ -GST sera would display better properties, as compared to traditional anti-peptide antibodies.

## Materials and Methods

**GST:** human  $\pi$ -GST was purchased from Sigma (product G 8642), and  $\mu$ -GST from Biotrin International, Cat. BIO58MU. The  $\alpha$  isoform (human) was a generous gift by Dr. G. Citro from our Institute.

**Multi-pin synthesis:** Peptides were synthesized on polypropylene pins according to the instructions of the Manufacturer (Chyron Mimotopes Pty Ltd, Australia).  $\alpha$ -Fmoc protected amino acids were purchased from Inalco, Milano. Syntheses were performed using  $N,N'$  diisopropylcarbodiimide

(DIPC) as coupling agent. The full sequence of  $\pi$ -GST was covered by synthesizing 51 10-mer peptides, with an overlapping of six residues (spacing: 4). Two additional peptides, linked respectively to pin 1 and pin 2, were simultaneously synthesized as positive and negative controls. Pin 1 bore the decapeptide EAGKDDYVKA (the same synthesized on pin 31), known to react with an anti-peptide antibody generated against the undecapeptide EAGKDDYVKAL prepared by conventional solid-phase synthesis. Pin 2 peptide was a negative control, i.e. AGAGAGAGAG.

**Conventional solid-phase synthesis:** Peptides were prepared by solid-phase synthesis by aid of a Dupont-Vega Peptide Synthesizer, Model 1000, by using the Boc/benzyl approach (Merrifield, 1963). Peptide NT was available from previous investigations (Evangelista *et al.*, 1991) and corresponded to the N-terminal dodecapeptide (pos. 2–13). Fragment B covered pos. 37–46, C pos. 53–62, D pos. 81–91, E pos. 113–123, and F, pos. 129–139. The last peptide (G) was the C-terminal fragment 194–206, as represented on pins 51 and 52. An additional peptide (X), selected from an unrelated protein, was added as a negative control. Four peptides (NT, D, E and G) were used for eliciting anti-peptide antibodies in rabbits, peptides B, C, D, E, F, G for preparing affinity ligands, according to the indications provided by the Multipin test. Peptides D, E and G were common to both experiments. The sequence of the eight peptides is reported in Table I.

Anti-peptide sera were prepared by immunization of rabbits with conjugates of KLH with peptides NT, D, E and G. The standard procedure of immunization and recovery of anti-peptide antibodies is that reported in a previous investigation (Evangelista *et al.*, 1992).

Two rabbits were immunized with commercial  $\pi$ -GST.

The ELISA assay on 96-wells microtiter plates was performed as previously described (Evangelista *et al.*, 1992). Wells were precoated with 0.5  $\mu$ g of protein or synthetic peptide.

ELISA on pins was performed according to the instruction of the Manufacturer (Chyron Mimotopes Pty Ltd, Australia). Briefly, the wells of a microtiter plate were filled with 150  $\mu$ l (at 5 mg/ml), of anti- $\pi$ -GST IgGs precipitated from rabbit immune sera by ammonium sulfate at 33% saturation.

Table I. Conventional Solid-Phase Synthetic Peptides used as Affinity Ligands for Isolating Specific Antibodies.

Name	Pos.	Sequence	Corresponding pins (approx.)
NT)	2–12	P P Y T V V Y F P V R G	--
B)	37–46	E T W Q E G S L K A	12 (LD2)
C)	53–62	L P K F Q D G D L T	16 (LD3)
D)	81–91	G K D Q Q E A A L V D	23 (LD4)
E)	113–123	E A G K D D Y V K A L	31 (LD5)
F)	129–139	K P F E T L L S Q N Q	35 (LD6)
G)	194–206	L A S P E Y V N L P I N G	51, 52 (LD8)
X)		E M T D I F A N A G N	--

All peptides but NT and X were selected from the sequence of  $\pi$ -GST on the basis of the high reactivity of pins with rabbit antisera, and prepared by conventional solid-phase synthesis. Peptides corresponding to the first and seventh reactivity peaks (pins 6–7 and 42–43) were not synthesized because of their high sequence homology with related sequences in  $\alpha$ -GST. X was a control peptide not related to GST.

After deprotection and purification, peptides were coupled to Sepharose and used for fractionating subsets of anti- $\pi$ -GST antibodies.

The highly hydrophilic peptides D and E, the C-terminal fragment G, and the N-terminal 12-residue peptide NT were additionally employed for generating antipeptide antibodies.

tion, and allowed to react for 60 minutes with the fully deprotected pin-bound peptides. Pins were then washed several times in a tray with PBS containing 0.2% Tween 20, 0.5% BSA, then reacted in the wells of a second microtiter plate with 150  $\mu$ l of a 1:2000 dilution of the second antibody (Goat antirabbit IgG-peroxidase). After 60 minutes, pins were washed again and allowed to react, in the wells of a third microtiter plate, with 150  $\mu$ l of substrate (*o*-phenyldiamine and hydrogen peroxide). The reaction was stopped after 5 minutes by addition of 50  $\mu$ l of 4 N sulfuric acid, and the absorbances at 492 nm measured in a microtiter reader (Immunella Mod. 990, GDV, Italy).

Conventional Sepharose-peptide immunoadsorbents were prepared by linking Sepharose-EAH to the synthetic peptides by aid of glutaraldehyde or, alternatively, by using COOH-activated Sepharose. The methods have been extensively reported in previous publications (Chersi *et al.*, 1991). The isolation of specific anti- $\pi$ -GST antibody subsets from the sera was obtained by slowly passing immune IgG samples (1.5 ml, at 10 mg/ml) through small 0.5 x 3 cm columns, each packed with a single immunoadsorbent. Columns were washed with PBS, then adsorbed proteins eluted

from each individual column by 0.2 M glycine-HCl buffer pH 2.6, immediately neutralized and dialyzed against PBS. For all chromatographies, a small 0.5 x 1 cm column of Seph-X was used as a pre-column for minimizing aspecific adsorptions.

An additional aliquot of immune IgGs was run however through the whole set of columns, and the depleted IgG sample used as a control.

## Results

For isolating specific anti- $\pi$ -GST antibody subsets from anti- $\pi$ -GST immune sera, we first identified the main sequential epitopes within the protein by synthesizing, by aid of the Multi-pin synthesis, 51 overlapping decapeptides (spacing: 4), covering the whole sequence of the protein, and then by testing their reactivity in ELISA with total anti- $\pi$ -GST rabbit IgG.

Several major reactivity peaks were detected (Fig. 1). This pattern should be compared with the hydrophilicity indexes for the same decapeptides, (Hopp and Woods, 1981), as reported in Fig. 2. The main reactivity peaks corresponded to pins 6–7, 11–12, 16–17, 23–24, 31–32, 34–35, 42–43, and 51–52 (Table II). Notably, pins 3 and 4, bearing the hydrophobic N-terminal fragment (NT), previously used as immunogen for producing antipeptide antibodies, exhibited very low activity. The highly hydrophilic amino acid stretches 81–90 (pin 23) and 113–126 (pins 31 and 32), but not 97–106 (VEDLRCKYIS, pin 27), corresponded to high activity peaks. Also the hydrophobic C-terminal segment of the protein (pins 51 and 52), resulted fairly active.

We assayed first whether pin-bound peptides could be used as affinity ligands to fractionate subsets of anti- $\pi$ -GST antibodies directed to the different immunogenic segments of the protein, using the approach suggested by other authors (Tribbick *et al.*, 1991). However, the total amount of protein recovered, from each set of pins, was quite low (0.01 to 0.04 mg), this suggesting that bound antibodies had been only partially released. Sonication (10 minutes at 40 °C, in PBS) resulted in a complete removal of IgG from the polyethylene rods, but antibodies resulted devoid of any activity.

Several peptides identified as epitopes were then prepared by conventional solid-phase synthesis (Table I), and then linked to Sepharose-EAH

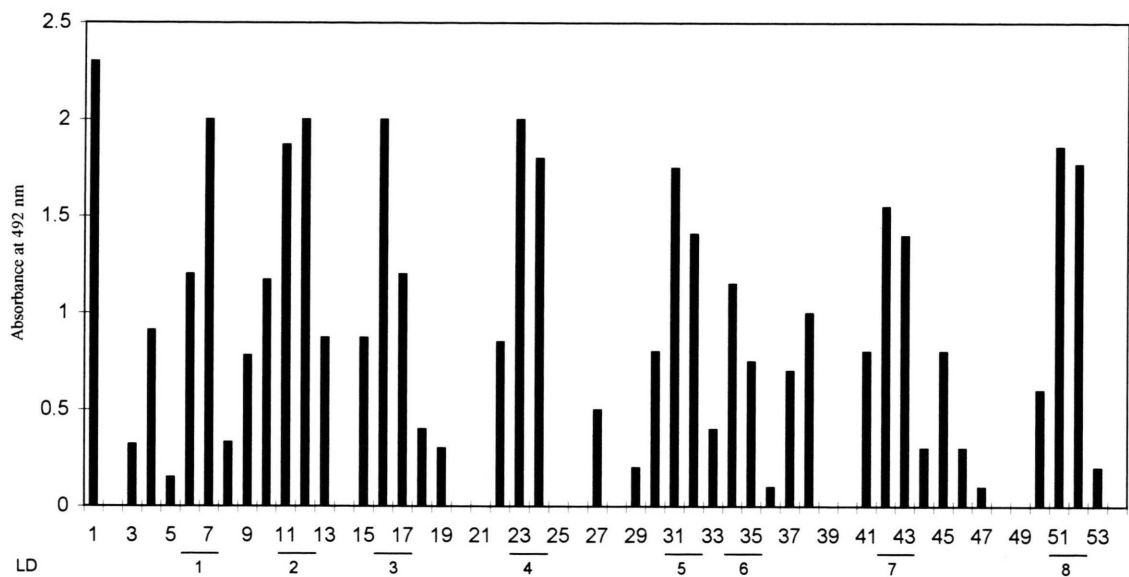


Fig. 1. Reactivity of pin-bound peptides with anti- $\pi$ -GST IgGs, as determined with a modified immunoassay. Pin 1 beared the decapeptide EAGKDDYVKA (same as pin 31), but was reacted with antipeptide antibody anti-E (EAGKDDYVKAL), and served as the positive control. Pin 2 beared the 10-mer peptide AGAGAGAGAG and served as the negative control. Reactive epitopes are underlined (LD: linear determinants), and numbered from 1 to 8. The test was performed in duplicates. The sequence of peptides bound to reactive pins is reported in Table II.

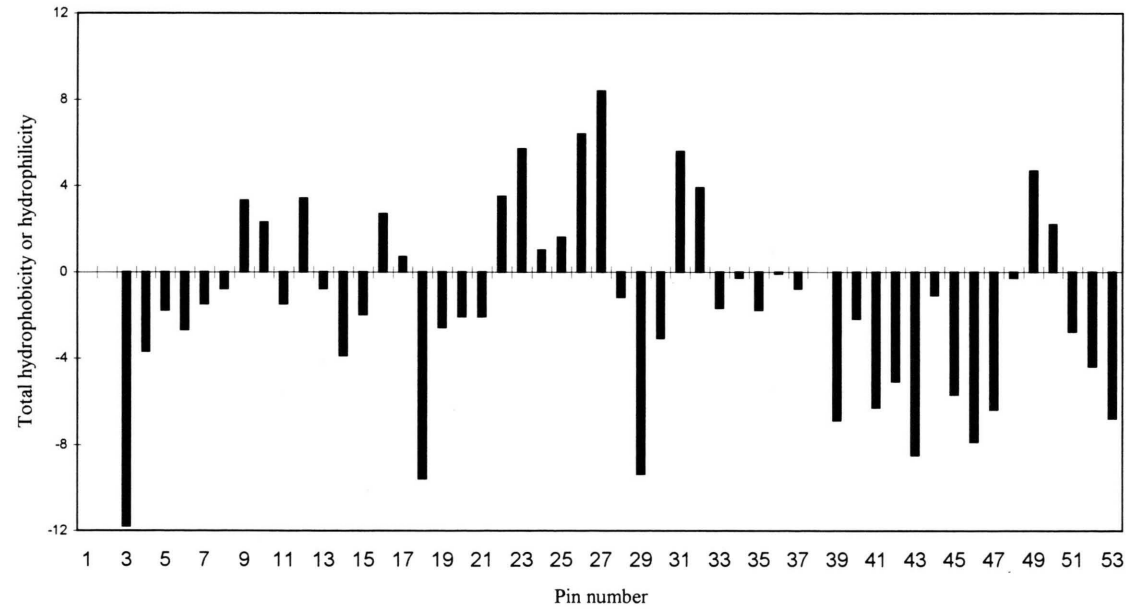


Fig. 2. Hydrophilicity profile of  $\pi$ -GST as calculated for each of the 51 decapeptides (Hopp and Woods,1981). The number reported is the sum of the individual hydrophilicity values of each of the ten amino acids constituting the decapeptide. To allow comparison of indexes with the activity of the pin-bound decapeptides with the antiserum (Fig. 1), positions 1 and 2 have been left free.



Table II. Sequences of Pin-Bound Peptides Exhibiting Reactivity in Elisa with Rabbit Anti- $\pi$ -GST IgGs.

Name	Pin numbers	Sequence	Pos.	Synth. peptides
LD 1	6	GRCAALRMLL	13–22	
	7	ALRMLLADQ G	17–26	
LD 2	11	VVTVETWQEG	33–42	<b>B</b>
	12	ETWQEGSLKA	37–46	
LD 3	16	LPKFQDGLT	53–62	<b>C</b>
	17	QDGLTLYQS	57–66	
LD 4	23	GKDQQEAAALV	81–90	<b>D</b>
	24	QEAAALVDMVN	85–94	
LD 5	31	EAGKDDYVKA	113–122	<b>E</b>
	32	DDYVKALPGQ	117–126	
LD 6	34	GQLKPFETLL	126–135	<b>F</b>
	35	PFETLLSQNQ	130–139	
LD 7	42	LDLLL I HEVL	157–166	
	43	L I HEVLAPGC	161–170	
LD 8	51	FLASPEYVNL	193–202	<b>G</b>
	52	PEYVNLPIG	197–206	

LD is used as abbreviation for Linear Determinant.

In **bold** letters (last column), the synthetic peptides B, C, D, E, F and G, prepared by conventional solid-phase synthesis, that approximately correspond to the active epitopes. Their sequence is reported in Table I.

or COOH-activated Sepharose. However, the fragments covering pos. 13–22 (LD1) and 157–170 (LD7) were not synthesized because of their high sequence homology with corresponding amino acid stretches in  $\alpha$ -GST.

Aliquots of IgG were loaded on the individual columns each filled with a different immunoadsorbent. Adsorbed antibodies were then eluted by 0.2 M glycine-HCl buffer, pH 2.6, immediately neutralized and dialyzed. An additional sample was run subsequently on the whole set of columns, connected tail-to-head. “Exhausted” IgG were collected for controls.

In a typical experiment, using aliquots of 15 mg of IgG of rabbit #1, the amount of affinity-purified antibody obtained from each column ranged from 0.12 mg (from Seph-F column), to 0.28 mg (from Seph-E column) (data not shown). A little amount of protein (0.08–0.10 mg) was also adsorbed by the pre-columns packed with Seph-X, in spite of the fact that peptide X was not related to  $\pi$ -GST. This protein sample, however, resulted completely inactive. The amounts of protein recovered from columns by loading IgGs of rabbit #2 were substantially comparable.

Three of the above mentioned peptides, plus peptide NT, corresponding to the amino terminal fragment, as available from previous investiga-

tions, (Evangelista *et al.*, 1991), were additionally used as immunogens for eliciting anti-peptide antibodies. Both fragments D and E represented quite hydrophilic domains in  $\pi$ -GST (Fig. 2), while peptide G corresponded to the C-terminal segment of the enzyme.

Antibodies were raised in rabbits using KLH-peptide complexes, as described under “Methods”. Immunoglobulins were precipitated from immune sera by ammonium sulfate at 33% saturation, and anti-peptide antibodies purified by affinity chromatography. The yield ranged from 0.6 to 1.5 mg antibody / 10 ml serum. Surprisingly, the response to peptide D was very weak, either when it was coupled to KLH (rabbit # 3), or to BSA (rabbit # 4).

All antibody samples obtained by the two approaches, suitably diluted to the same protein concentration (20  $\mu$ g/ml), were then tested with an ELISA on microtiter plates, precoating the wells with the three common isoform of GST, i.e.  $\pi$ ,  $\mu$ ,  $\alpha$ , as well as with the free peptides. The original IgG preparation, and the “exhausted” IgG sample recovered after the run through the 8 different columns, were tested simultaneously as a control. The data, partly reported in Figs 3 and 4, can be briefly summarized as follows:

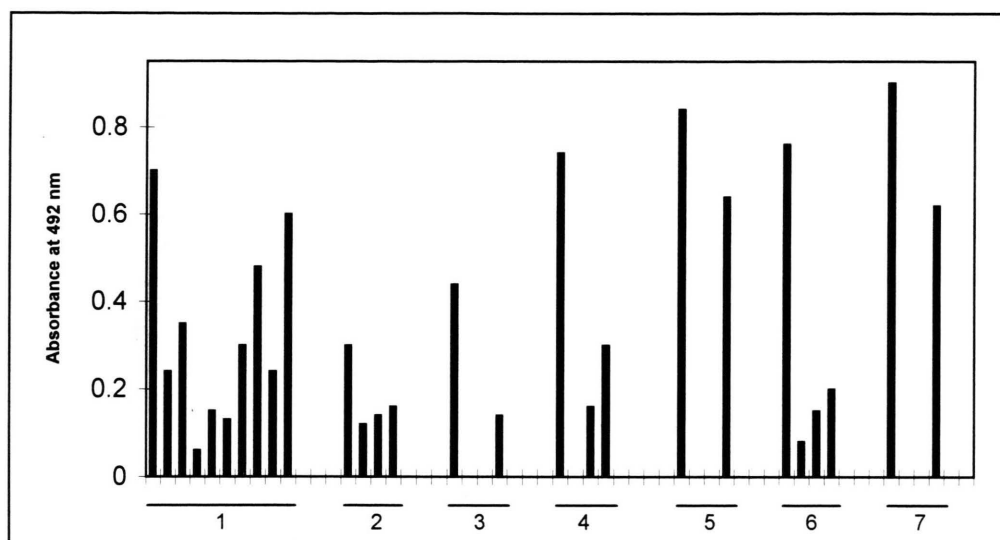


Fig. 3. Reactivity in ELISA of the original anti- $\pi$ -GST immunoglobulin preparation (total IgG) and of antibodies isolated by Sepharose-peptide immunoabsorbents, with the common isoforms of glutathione transferase  $\pi$ ,  $\mu$ , and  $\alpha$ , and with peptides used for immunodepletion.

Wells of microtiter plates were precoated with 0.5  $\mu$ g of antigens. Antibodies were diluted to the same protein concentration, (20  $\mu$ g/ml), and used in the amount of 50  $\mu$ l. Preimmune rabbit IgG was tested as a control, and the background values (range: 0.060–0.140) subtracted. The test was performed in triplicates.

For total anti- $\pi$ -GST immunoglobulins (1), the antigens are, in the order,  $\pi$ -GST,  $\mu$ -GST,  $\alpha$ -GST, and then peptides NT, B, C, D, E, F, and G, in the order. For antibodies subsets eluted from affinity columns (Seph-B to Seph-G, in the order, from 2 to 7), only 4 antigens are reported, i.e., the three isoforms of the enzyme  $\pi$ ,  $\mu$ , and  $\alpha$ , and then the corresponding peptide, at the fourth position. No one of the samples reacted with other fragments of the protein, except that eluted from Seph-C, which appeared to recognize also peptide B, and that eluted from Seph-G, which reacted also with peptide E (data are reported under "Results", and not shown). The binding values of the IgG sample after the run through the eight columns are summarized in the text, and not reported here for simplicity.

1) The original anti- $\pi$ -GST IgG preparation did not bind to peptide NT, as predictable from the ELISA on pins, and surprisingly very little to peptide B, in spite of the fact that in the preliminary experiment, pins 11 and 12 bearing this fragment were apparently quite reactive. The reactivity to peptides C, D, E, F and G (the C-terminal fragment) was confirmed. The crossreactivity with the  $\alpha$  and  $\mu$  isoforms of the enzyme was in the order of 50% and 35%, respectively.

2) The exhausted IgG sample, after the run through the whole set of columns, exhibited no residual activity on the peptides, but obviously still reacted with the native enzyme, this activity being due to antibodies directed against linear determinants that were not investigated (LD-1 and LD-7), or against conformational determinants and minor linear epitopes. The crossreactivity with  $\alpha$ -GST

and  $\mu$ -GST resulted substantially unmodified (data not shown in the figure).

3) Proteins eluted from Seph-X and Seph-NT were devoid of activity (data not shown), and those recovered from Seph-B scarcely reactive. Most antibodies eluted from the other columns recognized specifically only the corresponding peptide, but antibodies from Seph-C crossreacted partially (30%) with peptide B, these from Seph-G, with peptide E (30%) (data not shown).

In all samples, the crossreactivity with the  $\mu$  isoform was completely abolished, except in antibodies isolated by aid of Seph-F column (9% crossreactivity). The binding to the  $\alpha$  isoform completely disappeared in three samples, (Abs from Seph-C, Seph-E, Seph G), but was still measurable, although reduced, in two others (Abs from Seph-D: 15%; Abs from Seph-F: 12%).

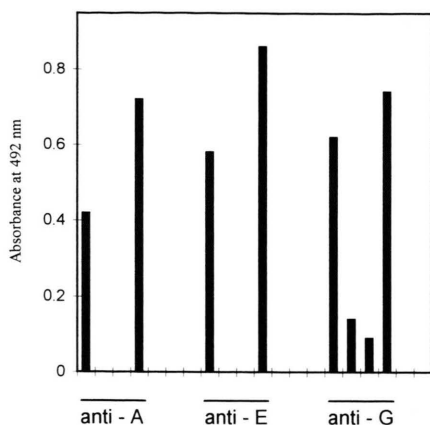


Fig. 4. Binding of anti-peptide antibodies anti-NT, anti-E, and anti-G, to  $\pi$ -GST,  $\mu$ -GST,  $\alpha$ -GST. Peptides NT, D, E, G, and the unrelated peptide X, were all used as controls. Only the binding to the cognate peptide are reported in the figure, at the fourth position, as no one of the anti-peptide antibodies displays crossreactivity with any other fragment. The assay was performed under the same conditions as in the previous experiment.

4) Anti-peptide antibodies anti-NT, anti-E, and anti-G displayed high affinity for the immunogen (the peptide), and were moderately active on the protein. The first two anti-peptide antibodies did not display any crossreactivity with other isoforms of the enzyme, but anti-G partially crossreacted with both  $\mu$ -GST (22%) and  $\alpha$ -GST (14%).

## Discussion

The ELISA tests on pins identify eight major linear immunogenic epitopes in  $\pi$ -GST. The stretch characterized by the highest hydrophilicity value (pins 26 and 27, Fig. 2), was not recognized as an epitope by total polyclonal anti- $\pi$ -GST sera.

The N-terminal fragment, bound to pins 3 and 4, was not recognized by anti- $\pi$ -GST antibodies, being thus either not immunogenic or buried in the interior of the intact molecule. This result was anticipated by other investigators (Hosoda *et al.*, 1990).

There was only a major epitope (pins 42 and 43, LD7), corresponding to amino acids 157–170, in the long hydrophobic stretch 140–192. Also a mouse monoclonal antibody had been found to recognize a not well-defined epitope located in this long segment (Hosoda *et al.*, 1990).  $\alpha$ -GST and  $\pi$ -GST however display a high sequence homology

in this domain, thus this region was considered unsuitable for the purpose of our investigation.

The pin's technique was reliable and fast as far as it concerned the analytical localization of immunogenic determinants. It failed however when applied to preparative antibody fractionation and isolation. On the contrary, the subsets of antibodies eluted from Sepharose-peptide immunoadsorbents were obtained fully active and in reasonable amounts. Most antibodies reacted selectively with the peptide used for depletion and with the protein. The crossreactivity with the  $\mu$  isoform, which was in the order of 35% in the original anti- $\pi$ -GST preparation, was completely abolished in all antibody samples but one. Crossreactivity with  $\alpha$ -GST (approx. 50%) was abolished or decreased in all samples. Antibodies eluted from Seph-D and Seph-F however still reacted in ELISA with  $\alpha$ -GST, although at a lower extent. For this first antibody, the crossreactivity with the  $\alpha$  isoform is easily explained by considering the close sequence homology of the two protein molecules in the region 80–92.

Peptide D, either linked to KLH or BSA, did not elicit antibodies in rabbits. It is likely that this fragment was scarcely immunogenic because of the occurrence of a closely related sequence in one of the rabbit glutathione transferases. The same fragment, however, was recognized as a determinant by antibodies elicited by the native protein, perhaps as a part of a complex conformational epitope.

It is noteworthy that antibodies against synthetic peptides NT, E, and G apparently display lower affinity for GST than antibodies fractionated from the polyclonal anti- $\pi$ -GST sera by aid of affinity ligands. The difference in activity might simply reflect a residual partial contamination of some antibody preparations by extraneous proteins. In few words, same sample might have achieved a lower purity than others, and thus display an apparent lower binding affinity. Thus, the comparison of the ratio:

reactivity on the peptide / reactivity on the protein

and the extent of crossreactivity with other isoforms, might then be the best parameter for selecting anti- $\pi$ -GST antibodies to be used for further investigations.

The binding data suggest that antipeptide antibodies tendentially display in ELISA higher affinity for the peptide (the immunogen) than for the protein. This low reactivity might be due to the fact that in the native enzyme, the segments mimicked by synthetic peptides might assume a rigid conformation, thus preventing antipeptide antibodies from reacting (Westhof *et al.*, 1984); subsets of antipeptide antibodies recognizing this conformation might in fact represent only a small fraction of the total antibody population (Nyman *et al.*, 1983; Chersi and Houghten, 1984; Di Modugno *et al.*, 1995). In addition, the sole binding and recognition site of antipeptide antibodies within the protein is restricted to a short ten-residue linear segment.

Antiprotein antibodies, on the contrary, seem to be more reactive with the protein, than with the peptide used as affinity ligand. It is likely that they recognize in the protein several amino acid stretches forming a conformational determinant. The binding of an antiprotein antibody to a peptide, in fact, as in affinity chromatography, does not imply that this sequence alone is necessarily the complete epitope. It has been reported that occasionally monoclonal as well as polyclonal antiprotein antibodies react independently with two,

sometimes even three, structurally unrelated synthetic peptides, corresponding to contiguous as well as not adjacent amino acid stretches of the protein used as immunogen (Chersi *et al.*, 1991; Georges *et al.*, 1993; Cianfriglia *et al.*, 1995).

Finally, it should be added that the reactivity of antibodies with  $\pi$ -GST on microtiter plates might not correspond to that in solution: in the ELISA assay, protein molecules occasionally undergo a partial denaturation with exposure of regions that are originally masked or buried. Under such conditions, antipeptide antibodies might now bind to the macromolecule. On the contrary, the reactivity of antiprotein antibodies with the protein might be higher in solution than in ELISA, as the integrity and precise conformation of the epitope is often necessary for the complete recognition of the binding site.

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