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Expression of human glutathione *S*-transferases in *Saccharomyces cerevisiae* confers resistance to the anticancer drugs adriamycin and chlorambucil

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Adaptation and resistance to chemicals in the environment is a critical part of the evolutionary process. As a result, a wide variety of defence systems that protect cells against chemical insult have evolved. Such chemical resistance mechanisms appear to play a central role in determining the sensitivity of human tumours to treatment with chemotherapeutic drugs. The glutathione *S*-transferases (GST) are important detoxification enzymes whose over-expression has been associated with drug-resistance. In order to evaluate this possibility we have expressed the human Alpha-class and Pi-class GST cDNAs that encode GST B_1B_1 and GST π in the yeast *Saccharomyces cerevisiae*. The expression of GST B_1B_1 or GST π resulted in a marked reduction in the cytotoxic effects of chlorambucil, a bifunctional alkylating agent, and an anthracycline, adriamycin. These data provide direct evidence that the over-expression of GST in cells can confer resistance to anticancer drugs.

INTRODUCTION

Life has evolved in a hostile environment. As a consequence Nature has devised defence mechanisms to protect cells against the various external pressures, such as chemical insult, oxidative damage and harmful radiation. Significant variations exist in the ability of different cells to withstand exposure to chemicals. In chemotherapy this variation can have important repercussions.

The ability of a cell to tolerate chemical stress may be an intrinsic characteristic of the cell or a feature that is acquired following chemotherapy. Intrinsic resistance is difficult to study and remains poorly understood. By contrast, significant advances have been made in understanding acquired resistance. This phenomenon can result from two types of phenotypic change: those that accentuate normal cellular defence processes or those that diminish or avoid the toxic effect of the drug through mutation or gene amplification (Stark & Calvert, 1986).

Drug-resistance remains the major limiting factor in cancer chemotherapy and understanding the mechanisms of this effect represents an essential step in improving cancer treatment. The discovery of P-glycoprotein, whose over-expression is associated with multidrug-resistance, represented a major advance in this area (Bradley *et al.*, 1988). However, this phenotype, which increases drug efflux, does not confer resistance to anticancer drugs such as alkylating agents, which are a major class of chemotherapeutic agent. Resistance to these agents must therefore involve other mechanisms.

There has recently been increasing interest in the possible role of chemical-detoxification enzymes, such as the glutathione *S*-transferases (GSTs; EC 2.5.1.18) in resistance towards these compounds (Hayes & Wolf, 1988; Wolf *et al.*, 1990). The cytosolic GSTs are a complex group of proteins which have been classified on the basis of sequence homology into three multigene families, termed Alpha, Mu and Pi (Mannervik & Danielson, 1988). These proteins have been intimately linked to the detoxification of environmental chemicals and carcinogens (Chasseaud, 1979; Sies & Ketterer, 1988), and have been shown to be over-expressed in normal and tumour cells following exposure to cytotoxic drugs (Wang & Tew, 1985; Adams *et al.*, 1985; Batist *et al.*, 1986; Cowan *et al.*, 1986; McGowan & Fox, 1986; Buller *et al.*, 1987; Evans *et al.*, 1987; Robson *et al.*, 1987; Wolf *et al.*, 1987; Lewis *et al.*, 1988; Smith *et al.*, 1989). They have also been shown to be over-expressed in carcinogen-induced rat liver preneoplastic nodules (reviewed by Faber, 1984). In all these cases the changes in GST expression are associated with a concomitant increased resistance to cytotoxic chemicals. The possibility that the GSTs are involved in the resistance mechanism was substantially strengthened by the finding that Alpha-class GST genes were amplified in Chinese-hamster ovary (CHO) cells made resistant to chlorambucil (Lewis *et al.*, 1988).

In order to obtain direct genetic evidence that GSTs are capable of conferring drug-resistance we have expressed cDNAs encoding GST π and GST B_1B_1, which are known to be expressed in human tumours (Di Ilio *et al.*, 1985; Kodate *et al.*, 1986; Carmichael *et al.*, 1988; Shea *et al.*, 1988; Moscow *et al.*, 1989), in the yeast *Saccharomyces cerevisiae* and determined whether this results in diminished sensitivity to cytotoxic drugs.

MATERIALS AND METHODS

Microbial strains and plasmids

The following microbial strains were used in this study: *Escherichia coli* strains DH1 (*F⁻recA1 gyrA96 thi-1 hsdR17 supE44λ*), JM101 [*thi supE* Δ(*proAB-lac*) F′ (*TraD*36, *proAb lacZΔM15 I^q*)], HB101 [*F⁻*, *hsdS20-*($r_B^-M_B^-$) *recA13 ara-14*

Abbreviation used: GST, glutathione *S*-transferase.

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proA2 lacY1 galk2 resp120 (*SM*^{R}) *xyl-5 mtl-1 supE44λ*] and *Saccharomyces cerevisiae* strain KY118 (*α, his3-Δ200 lys2-801*^{am} *ade2-101*^{oc} *trp*-1-*Δ1 ura3-52*). The yeast expression plasmid pMA56 (Ammerer, 1983) was constructed from the plasmid MW5-906 obtained from Ben Hall (Department of Genetics, University of Washington, Seattle, WA, U.S.A.). The yeast expression vector pVT100-U was a gift from David Thomas (Vernet *et al.*, 1987). Both these vectors contain the *ADC1* alcohol dehydrogenase promoter and give constitutive expression of the inserted DNA. However, they contain *TRP1* and *URA-3* as selectable markers respectively. Isolation of the human Alpha-class and Pi-class GST cDNAs has been described previously (Lewis *et al.*, 1988; Kano *et al.*, 1987). The Alpha-class GST cDNA encodes a protein described by Stockman *et al.* (1987) as the subunit of GST B_1B_1.

Media

E. coli was cultivated in L broth, containing 10 g of bactotryptone (Difco), 5 g of yeast extract (Difco) and 5 g of NaCl per litre of distilled water. In those *E. coli* strains possessing a plasmid, L broth was supplemented by 50 μg of ampicillin/ml. *S. cerevisiae* was cultivated in synthetic minimal medium, containing 6.7 g of Bacto-yeast nitrogen base without amino acids (Difco), 20 g of glucose (Difco), 1% (w/v) casein hydrolysate (casamino acids) (Difco) and 20 mg of adenine per litre of distilled water. When the pMA56 vector was used 20 mg of uracil was also added.

Recombinant DNA procedure

Vectors and recombinant plasmids were isolated from cultures of *E. coli* by using the alkaline-lysis method of Birnboim & Doly (1979). *Eco*RI fragments containing the complete coding region of Alpha-class GST or Pi-class GST were cloned into the plasmid pMA56 using the unique *Eco*RI cloning site. The Pi-class GST was also blunt-end-ligated into the *Pvu*II site of vector pVT100-U. After propagation in *E. coli* the isolated DNA was transfected into *S. cerevisiae* by using the lithium acetate method (Itoh *et al.*, 1983). Restriction nucleases and T4 DNA ligase were purchased from Bethesda Research Laboratories. DNA polymerase I Klenow fragment was purchased from Boehringer Mannheim. Restriction digestions and ligations were performed using the manufacturers' recommended conditions.

Preparation of yeast subcellular fractions

Yeast cultures (500 ml) were harvested in late-exponential phase by centrifugation at 5000 rev./min in a Sorvall RC-5B centrifuge for 10 min. The pellets were then resuspended in 0.01 M-potassium phosphate buffer, pH 7.4, containing 1.15% (w/v) KCl. These suspensions were then passed through a French press to disrupt the yeast cell wall (Duppel *et al.*, 1973). Subcellular fractionation was then carried out as described by Yoshida *et al.* (1974). The cell debris was removed by centrifugation at 8000 *g* for 10 min and the mitochondrial fraction was obtained by centrifugation at 12000 *g* for 20 min. The microsomal and cytosolic fractions were obtained by centrifugation at 205000 *g* for 90 min in a TFT 45.6 rotor in a Sorvall OTD65-B ultracentrifuge.

Western-blot analysis

Western blots were carried out by using a modified version of that described by Towbin *et al.* (1979) as previously described by Lewis *et al.* (1988). After SDS/PAGE and transfer to nitrocellulose, filters were washed twice in TBST buffer [50 mM-Tris/HCl buffer, pH 7.9, containing NaCl (0.15 M) and Tween 20 (0.05%, v/v)]. Filters were blocked for 1 h in TBST buffer containing BSA (3%, w/v) followed by two 10 min washes in TBST buffer. Specific GST antisera against B_1B_1 and Pi-class GST (Stockman *et al.*, 1987; Howie *et al.*, 1988) (diluted 1:500) were then added for 1 h, followed by four 15 min washes in TBST buffer. Filters were then incubated for 1 h with donkey anti-(rabbit IgG) antibody conjugated to horseradish peroxidase. After further washes in TBST buffer the bands were detected either by using 4-chloro-1-naphthol as peroxidase substrate or by autoradiography after labelling with 0.19 MBq of ^{125}I-Protein A.

Assays of GSH and GST activity

GST activity towards the substrate 1-chloro-2,4-dinitrobenzene was determined by the method of Habig *et al.* (1974). Peroxidase activity towards cumene hydroperoxide was determined in a coupled reaction by measuring the rate of NADPH oxidation (Paglia & Valentine, 1967). GSH concentration was determined by the method of Hissin & Hilf (1976).

Cytotoxicity assays

Assays were carried out on yeast cultures transformed with the Alpha-class and Pi-class GST expression vectors (as well as the control vector containing the Pi-class GST in the reverse orientation). Cells were grown to late-exponential phase and the cell numbers were determined with a haemocytometer. Cells (4×10^7) were then harvested and pelleted (at 3000 rev./min in a Beckman D6 centrifuge for 5 min) and resuspended in 1.96 ml of phosphate-buffered saline, pH 7.3 (Oxoid, Basingstoke, Hants., U.K.). GST expression in the cultures was then confirmed by measuring GST activity towards 1-chloro-2,4-dinitrobenzene. Test chemical, dissolved in 40 μl of dimethyl sulphoxide for chlorambucil or 40 μl of sterile distilled water for adriamycin, was then added. An equivalent concentration of dimethyl sulphoxide or water was added to relevant control cultures. Cells were then placed at 37 °C in an orbital shaker for 2 h. Cultures were then diluted, and 100 μl (200 cells) aliquots were plated in triplicate on to complete agar plates and incubated at 28 °C. Survival was determined by counting the colonies after 3 days.

GST-binding assay

In order to establish whether the Alpha-class GST or Pi-class GST could bind adriamycin, Alpha-class GST (150 μg) or mouse Pi-class GST (100 μg) was incubated in 200 μl of phosphate-buffered saline, pH 7.3, with ^{14}C-labelled adriamycin (100 nmol; 125000 d.p.m.) (Amersham International) overnight at 4 °C. The mixture was then applied to a Protein PAK Gloss SW (Waters) gel-filtration h.p.l.c. column. Samples were eluted with 20 mM-sodium phosphate buffer, pH 6.5. The GST and adriamycin peaks were monitored at 280 nm. Fractions (1 ml) of the eluate were collected in scintillation vials and radioactivity content was determined by liquid-scintillation counting.

RESULTS

Expression of single human GST enzymes in yeast

Transformation of yeast cells with the pMA56 vector containing full-length Pi-class GST or GST B_1B_1 cDNAs gave high levels of GST expression, up to 0.5% of total yeast protein in each case. Western-blotting experiments, with antisera raised against GST B_1B_1 and GST π, and with the human enzymes as standards, showed that the expressed GSTs were of the correct molecular size and were localized predominantly in the yeast cytosolic fraction (Fig. 1). No protein that cross-reacted with anti-(GST B_1B_1) IgG or anti-(GST π) IgG was seen in wild-type *S. cerevisiae* cells. The expressed GSTs were catalytically active towards compounds that are model GST substrates (Fig. 2) and, in agreement with studies on the purified enzymes, the cytosol that expressed Alpha-class GST B_1B_1 conjugated 1-chloro-2,4-di-

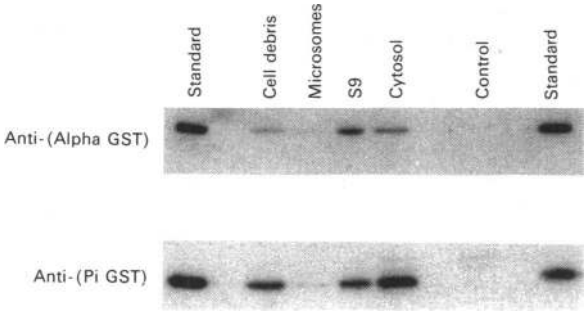

Fig. 1. Expression of Alpha-class and Pi-class GSTs in *S. cerevisiae*

Yeast cells were transformed with the pMA56 vector containing Pi-class GST cDNA or Alpha-class GST B_1B_1 cDNA as described in the Materials and methods section. Cells were harvested in late-exponential growth, and protein samples (25 μg) from various yeast subcellular fractions were separated by SDS/PAGE. The samples were then analysed by Western-blot analysis with antibodies to GST B_1B_1 and Pi-class GST. Subcellular fractions were prepared as described in the Materials and methods section: cell debris, 12000 *g* pellet; S9, ~ 12000 *g* supernatant; microsomes, 205000 *g* pellet; cytosol, 205000 *g* supernatant. The Pi-class GST and Alpha-class GST B_1B_1 standards were isolated as described previously (Stockman *et al.*, 1987; Howie *et al.*, 1988). No cross-reacting bands were observed in the cytosol of the wild-type yeast or yeast containing Pi-class GST cDNA inserted into the pMA56 vector in the reverse orientation (control).

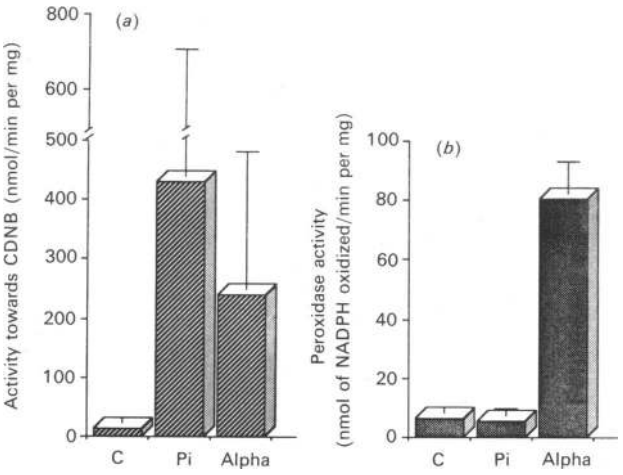

Fig. 2. GST activity in cytosolic fractions of *S. cerevisiae*

Yeast cells were transformed with the pMA56 vector containing the cDNA for human Alpha-class GST B_1B_1, Alpha-class GST, Pi-class GST or Pi-class GST in the reverse orientation [control (C)]. In this experiment the GST activity was measured in the cytosolic fraction with either 1-chloro-2,4-dinitrobenzene (CDNB) (*a*) or cumene hydroperoxide (*b*) as substrate. GST-mediated conjugation of 1-chloro-2,4-dinitrobenzene with GSH was assayed at 30 °C by the method of Habig *et al.* (1974). Peroxidase activity was measured with cumene hydroperoxide as substrate and was determined in a coupled reaction by measuring the rate of NADPH oxidation (Paglia & Valentine, 1967). The data represent the means ± S.D. for 22 determinations with 1-chloro-2,4-dinitrobenzene and for six determinations with cumene hydroperoxide.

nitrobenzene and possessed peroxidase activity towards the substrate cumene hydroperoxide. By contrast, the cytosol that contained human GST π metabolized 1-chloro-2,4-dinitrobenzene but not cumene hydroperoxide (Mannervik & Danielson, 1988). A relatively large variability in the activity towards 1-chloro-2,4-dinitrobenzene was measured among different cultures, the highest activities being 1000 nmol of 1-chloro-2,4-dinitrobenzene conjugated/min per mg of protein (i.e. 1000 units). This represents a 100–200-fold higher rate than the background and is similar to that measured in human liver cytosol. However, in most experiments the activity towards 1-chloro-2,4-dinitrobenzene usually ranged between 350 and 600 units for cells expressing GST π and between 100 and 250 units for yeast cells expressing GST B_1B_1.

Alpha class GSTs have been shown to be over-expressed in cell lines made resistant to the anticancer drug chlorambucil (Robson *et al.*, 1987; Lewis *et al.*, 1988), and Pi-class GSTs have been reported to be over-expressed in adriamycin-resistant cell lines (Batist *et al.*, 1986; Cowan *et al.*, 1986). For this reason cytotoxicity assays were carried out on the transfected *S. cerevisiae* cells with these compounds. A marked increase in resistance to both compounds was consistently measured in cultures expressing either of the GSTs (Figs. 3*a* and 3*b*). No difference in sensitivity between the wild-type KY118 strain and the strain containing the GST cDNAs in the inverse orientation was observed. This result indicated that the presence of the plasmid in the transfected cells was not affecting sensitivity. Fig. 3(*a*) shows that, when a dose of adriamycin was employed that killed almost all of the control cells, up to 40% of cells expressing GST B_1B_1 and 25% of those expressing GST π survived, the change in sensitivity being 16.0-fold and 10.0-fold respectively. With chlorambucil at a dose of 500 μg/ml only 3% of the control cells survived whereas 15% and 27% of the cells containing Pi-class and Alpha-class GST respectively survived.

Survival curves were constructed on five separate occasions, and, although there was some variability between experiments, in all five cases the cells expressing the GSTs were significantly more drug-resistant. With adriamycin at a dose of 80 μg/ml the resistance increased 2.3–10.0-fold and 3–16.0-fold for cultures expressing Pi-class GST and GST B_1B_1 respectively. In cultures exposed to chlorambucil at a dose of 600 μg/ml the resistance increased 2.0–5.2-fold and 2.0–8.0-fold respectively. This fluctuation could be attributed to the variation in the level of GST expression between experiments. Indeed, there appeared to be a linear relationship between the amount of Alpha-class GST B_1B_1 expressed and the degree of resistance to chlorambucil at 600 μg/ml (Fig. 4).

Simultaneous expression of GST B_1B_1 and GST π

In view of the protective effects of GST B_1B_1 and GST π, it was of interest to establish whether an even higher level of resistance could be obtained in cells expressing Alpha-class and Pi-class GST simultaneously. These cytotoxicity experiments were carried out with adriamycin (Fig. 5). The GST π cDNA was cloned into pVT100-U vector, which uses the same *ADC1* promoter but *URA-3* as a selectable marker. Yeast cells were transformed simultaneously with this and the pMA56 vector containing the Alpha-class GST. The resultant colonies were shown to express both GST B_1B_1 and GST π. In some cases both GST subunits were expressed to a similar extent, but in others there was preponderance of one enzyme. GST activity towards 1-chloro-2,4-dinitrobenzene was found to be higher in the strains expressing both GSTs (Fig. 5*a*). A marked decrease in adriamycin toxicity was seen in cells expressing both GST subunits, with 70% of the cells surviving at 80 μg/ml relative to 48% and 45% in cultures expressing GST Alpha or GST Pi alone. In a repeat experiment survival in the cells expressing both GST subunits at this adriamycin dose was 77%. In cultures expressing only the Pi-class GST or GST B_1B_1 survival was 35% and 39% respectively.

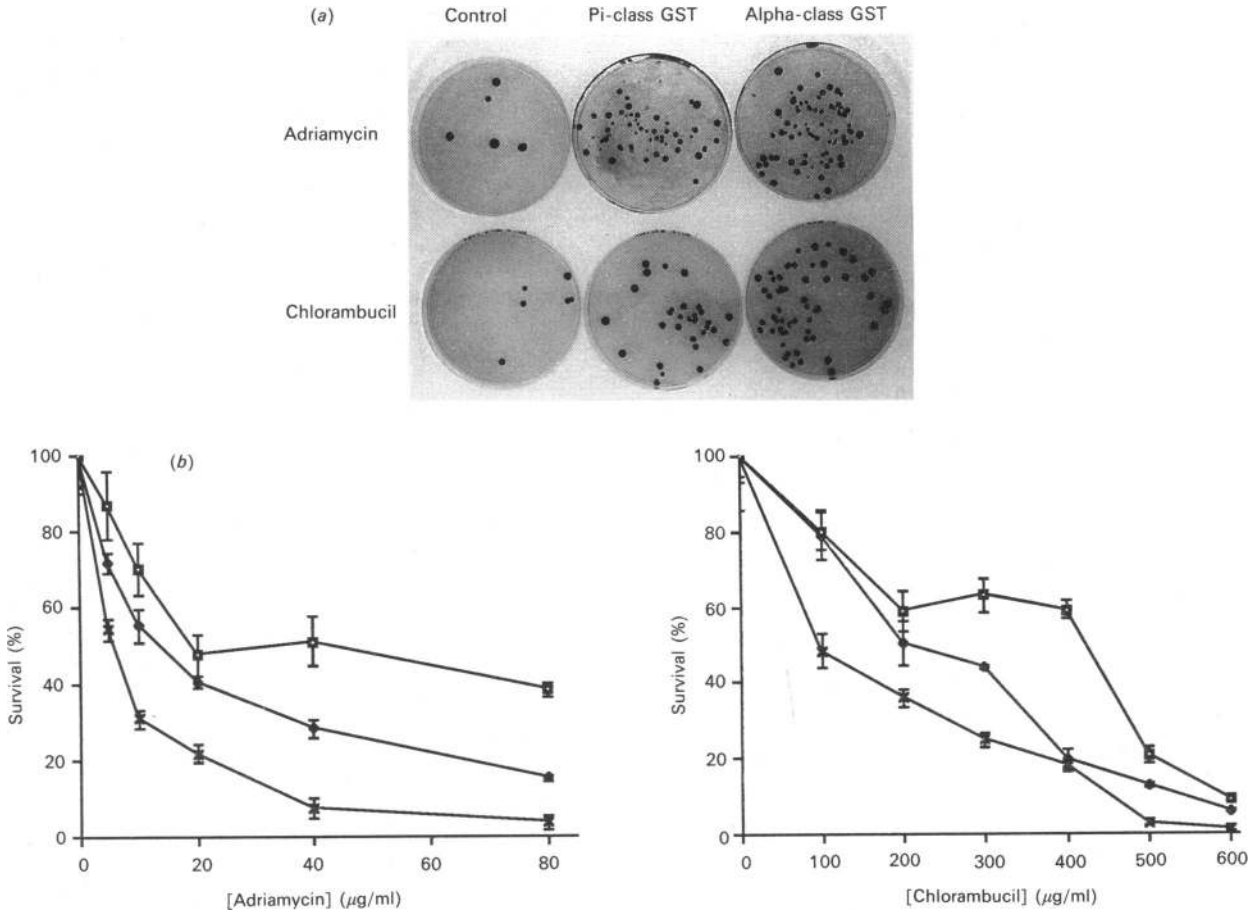

Fig. 3. Sensitivity of *S. cerevisiae* expressing Alpha-class GST and Pi-class GST to chlorambucil and adriamycin

(*a*) Agar plates showing the number of colonies obtained when yeast cells, transformed with pMA56 containing Pi-class GST cDNA in the inverse orientation (control), Pi-class GST cDNA or Alpha-class GST B_1B_1 cDNA, were exposed to either chlorambucil or adriamycin. The concentrations of adriamycin and chlorambucil used in the experiment were 80 μg/ml and 500 μg/ml respectively. Two-hundred cells were plated out. The plates show representative samples. The variation between samples was less than 10%. (*b*) Effect of drug dose on cell survival. In these experiments cultures containing Pi-class GST (◆) had a GST activity of 597 units and those containing Alpha-class GST B_1B_1 (■) 103 units. × represents control cultures. The experiments were carried out in triplicate and values are shown as means ± S.D. The data are expressed as percentages of the survival obtained in cultures where no drug was added.

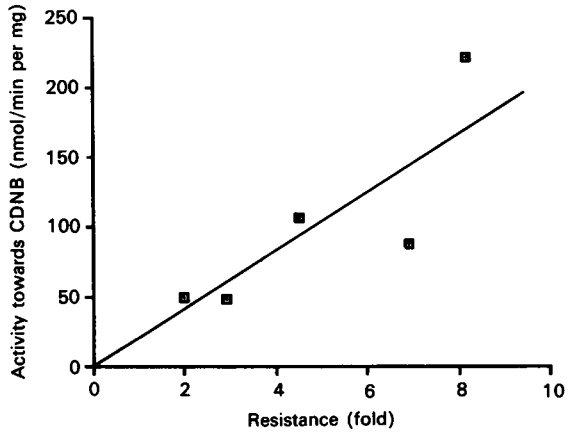

Fig. 4. Relationship between the level of GST B_1B_1 expression and resistance to chlorambucil

Cytotoxicity assays were carried out at a chlorambucil concentration of 600 μg/ml. Measurements of activity towards 1-chloro-2,4-dinitrobenzene (CDNB) were carried out as described in the legend to Fig. 3 and in the Materials and methods section. Fold resistance was determined from the number of survivors in cells expressing GST B_1B_1 relative to controls.

Role of GSH in GST-mediated resistance to chlorambucil

Many functions have been ascribed to GSTs that could account for their protective role. These include drug detoxification through conjugation with GSH, drug sequestration, DNA repair and possibly a function as stress-response proteins (Adams *et al.*, 1985; Carr *et al.*, 1986). In order to gain some insight into their mechanism of action, and whether GSH can be implicated in the protection process, cells were treated with the GSH-depleting agent buthionine-*SR*-sulphoximine. This treatment resulted in a significant depletion of cellular GSH in all the cells tested (Fig. 6), and was accompanied by an increase in the sensitivity to the cytotoxic effects of chlorambucil. This effect was particularly marked at the higher drug doses. In two separate experiments buthionine-*SR*-sulphoximine treatment reversed the GST-mediated resistance to this compound over the entire drug concentration range (Fig. 6*a*). These results are consistent with the hypothesis that GSTs utilize GSH in the protective mechanism. In view of the involvement of GSH in chlorambucil-resistance, it was feasible that the GSTs may be acting indirectly by altering the cellular GSH concentrations. However, the GSH content in all the yeast strains was similar, being 24.9 ± 2.9, 23.0 ± 6.9 and 25.8 ± 9.7 nmol/mg of soluble protein for control cells and for cells expressing the Alpha-class and Pi-class GST respectively.

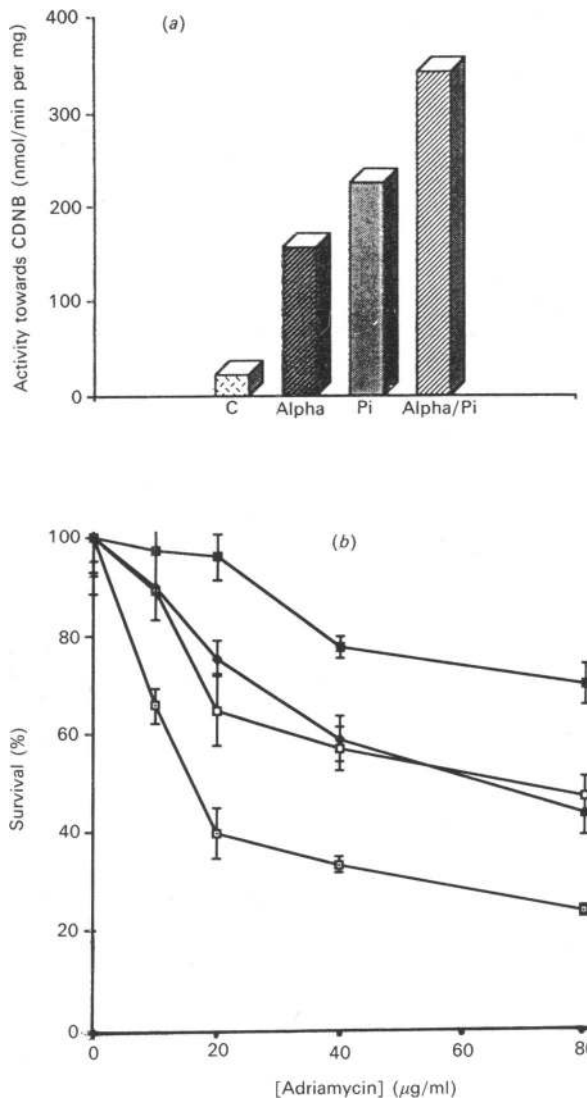

Fig. 5. Effect of concomitant expression of Alpha-class and Pi-class GSTs on the cytotoxicity of adriamycin

(*a*) GST activity in cultures used for the cytotoxicity assay measured with 1-chloro-2,4-dinitrobenzene (CDNB) as substrate: control cultures (C), cultures expressing the Alpha-class GST B_1B_1, cultures expressing Pi-class GST and cultures expressing both Alpha-class GST and Pi-class GST simultaneously. (*b*) Adriamycin-induced cytotoxicity on the yeast cultures described in (*a*): ⊡, control cultures; ◆, cultures expressing Alpha-class GST; □, cultures expressing Pi-class GST; ■, cultures expressing both Alpha-class GST and Pi-class GST simultaneously. Experimental details are given in the Materials and methods section. The values are shown as means ± S.D.

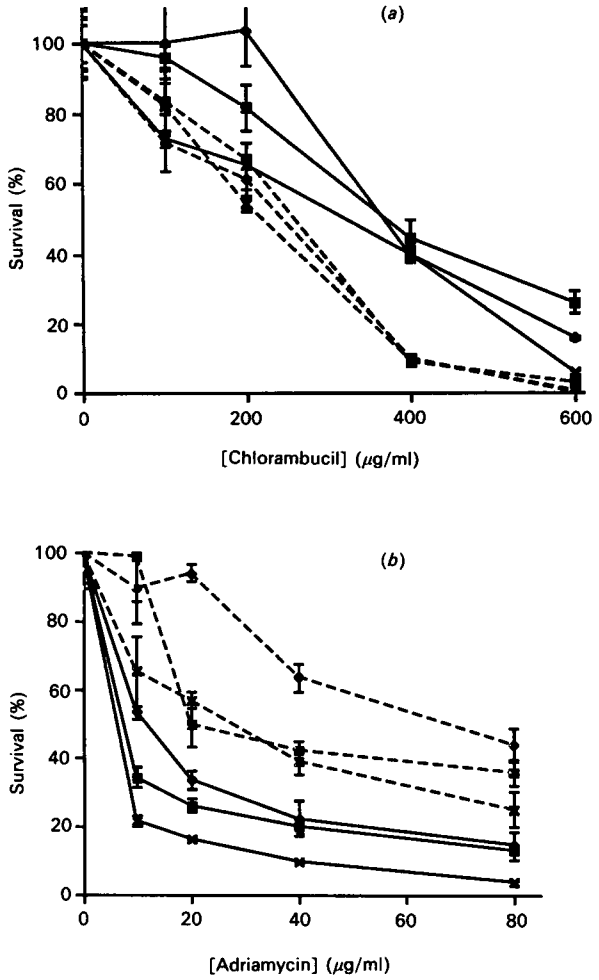

Fig. 6. Effect of buthionine-*SR*-sulphoximine treatment on the cytotoxicity of chlorambucil and adriamycin

Yeast cells were treated for 14 h with buthionine-*SR*-sulphoximine (10 mM) before being harvested. At this time cell samples were taken for determination of GSH (Hissin & Hilf, 1976). The cytotoxicity of adriamycin and chlorambucil was determined as described in the Materials and methods section. Buthionine-*SR*-sulphoximine treatment decreased GSH concentrations in control cultures and those expressing Alpha-class GST and Pi-class GST to 17%, 27% and 33% respectively of those measured in untreated cells. Broken lines are the results from cell cultures treated with buthionine-*SR*-sulphoximine. Continuous lines are from untreated cell cultures. ×, Control; ■, cultures expressing Alpha-class GST; ◆, cultures expressing Pi-class GST. The values are shown as means ± S.D.

Role of GSH in GST-mediated resistance to adriamycin

GSH depletion had surprising effects on adriamycin toxicity (Fig. 6*b*). In two separate experiments buthionine-*SR*-sulphoximine treatment significantly decreased the toxicity of adriamycin in all cultures, the toxicity being lowered 2–6-fold, depending on the adriamycin concentration. In spite of this effect, yeast cells expressing Pi-class GST were still more resistant than the wild-type cells to this compound. However, following GSH depletion, no difference in sensitivity was observed between control and GST-B_1B_1-expressing cells. It therefore appears that GSH is only partially involved in protection against adriamycin. In view of the drug-binding capacity of the GSTs, experiments were carried out to see whether adriamycin was sequestered by GST protein. Radioactive adriamycin was mixed with the GSTs and subjected to gel-permeation chromatography. However, no radioactivity became associated with the peak containing the GST protein, indicating that no binding had occurred (results not shown).

DISCUSSION

There are now many reports of over-expression of GST in tumour-cell lines resistant to cytotoxic drugs (see the Introduction), yet there is still only limited understanding of the role

of these proteins in this phenomenon. We show here that the GSTs can play a direct role in the resistance mechanism, a finding that is substantiated by the strong evidence implicating these proteins in acquired chemical resistance in a wide range of phylogeny, including plants, insects (reviewed in Hayes & Wolf, 1988) and bacteria (Arca *et al.*, 1988).

How GSTs confer resistance to the anticancer drugs is unclear. GST-mediated GSH conjugation may well play a role in the resistance to chlorambucil. GSH was identified as a potentially important factor in resistance to nitrogen mustards in pioneering studies almost 30 years ago (Hirono, 1960; Calcutt & Connors, 1963). In addition, it has been shown more recently that melphalan, another bifunctional alkylating agent, is a GST substrate (Dulik *et al.*, 1986). The reversal of the drug-resistance by GSH depletion supports this possibility and indicates that in the case of chlorambucil-resistance mechanisms such as drug sequestration are not involved.

The mechanism of GST-mediated resistance to adriamycin is more complex. This may well be related to the numerous possible mechanisms of action of this drug, which include activation to DNA-damaging agents, redox cycling, inhibition of topoisomerase II and effects on cell membranes (Kessel, 1989). GSH depletion appeared to influence adriamycin resistance mediated by the Alpha-class GST B_1B_1 but did not affect the resistance mediated by GST π, indicating that these proteins act by different mechanisms.

All the GST enzymes are able to bind toxins non-covalently. It is therefore possible that GST can protect against adriamycin by drug sequesteration. Neither GST B_1B_1 nor GST π had the capacity to bind this compound in such a manner, indicating that this is not part of the resistance mechanism.

Both the Alpha-class and Pi-class GSTs were effective in protecting against the cytotoxic effects of both adriamycin and chlorambucil; indeed, in yeast cells expressing both subunits a higher degree of resistance was observed. This is particularly interesting, as early studies indicated that adriamycin-resistance led to over-expression of GST π (Batist *et al.*, 1986; Cowan *et al.*, 1986) and that resistance to alkylating agents was often accompanied with the over-expression of Alpha class GST (Robson *et al.*, 1987; Buller *et al.*, 1987; Lewis *et al.*, 1988). More recent studies, however, have shown that both Alpha-class and Pi-class GST activities are elevated in tumour-cell lines resistant to alkylating agents (C. J. Wareing, J. D. Hayes, S. M. Black & C. R. Wolf, unpublished work). Also, in alkylating-agent-induced preneoplastic foci in rat liver, which are also drug-resistant (Faber, 1984), Alpha-, Pi- as well as Mu-class GSTs are over-expressed. These findings would be consistent with the involvement of all these proteins in resistance to alkylating agents. In any given situation the GST enzyme that is actually over-expressed may be determined by the cell lines used and the presence of necessary transcription factors.

The importance of the GSTs in drug-resistance in cancer patients is at present unclear. In an early study we found increased GST activity in tumour-cell lines derived from a patient following treatment with chlorambucil and *cis*-platinum (Wolf *et al.*, 1987). We have also some recent results that indicate a higher level of GST π expression in chronic lymphocytic-leukaemia patients exhibiting resistance to chlorambucil treatment (Holmes *et al.*, 1990).

Over-expression of the GSTs appears to be only one of many drug-resistance mechanisms. In most drug-resistant cell lines, as well as in preneoplastic foci, multiple changes resulting in the over-expression of many proteins occurs (reviewed in Wolf *et al.*, 1990). In preneoplastic nodules these multiple changes are often observed after a single dose of carcinogen, which indicates that the over-expression of these proteins is mediated by a common mechanism(s). Such a phenomenon could be explained by the permanent expression of a normally transient stress-response process. This would imply that over-expression of the GSTs may occur in certain circumstances as a consequence of chemical stress and may not be a requirement for drug-resistance. However, the data presented here, the consistent observation that GSTs are over-expressed in drug-resistant cells together with the extensive literature demonstrating their role in chemical detoxification provides strong evidence that in certain cases they are directly involved in the drug-resistance mechanism.

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