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Ricin B chain-containing immunotoxins prepared with heat-denatured B chain lacking galactose-binding ability potentiate the cytotoxicity of a cell-reactive ricin A chain immunotoxin

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Ricin B chain incubated at 37°C in the absence of lactose loses its ability to bind the galactose-containing protein, asialofetuin. Circular dichroism analysis of the B chain during thermal denaturation indicates that the loss of galactose-binding ability by the B chain correlates with limited unfolding of the molecule. As a result of this conformational change, disulfide bonds that are shielded from the solvent by the compact folded structure of the B chain become exposed and the chitobiosyl cores of both N-linked oligomannose chains become susceptible to cleavage by endoglycosidases. The heat-denatured B chain does not enhance the toxicity of a ricin A chain-containing rabbit anti-human immunoglobulin (RAHIg-A) to Daudi cells. However, when heat-denatured B chain is coupled to goat anti-rabbit immunoglobulin (GARIg), the resulting immunotoxin, GARIg-hdB, potentiates the killing of RAHIg-A-treated Daudi cells to an extent similar to that of an immunotoxin prepared with GARIg and native B chain. These results indicate that the native, galactose-binding structure of the B chain is not necessary to enhance the cytotoxicity of the cell-reactive A chain immunotoxin (IT-A) and suggests that regions of the B chain exposed by unfolding the molecule may mediate potentiation of cytotoxicity.

Introduction

The plant toxin, ricin, prepared from the castor bean *Ricinus communis*, consists of two disulfide-bonded polypeptide subunits (A and B chains). The A chain catalytically inactivates eukaryotic ribosomes and the B chain is a lectin that binds to galactose-terminating oligosaccharides present on the surface of most cells. Cell-bound ricin is internalized by endocytosis and the A chain is then translocated to the cytosol where it inhibits protein synthesis [1,2].

Immunotoxins prepared by coupling native ricin to monoclonal antibodies directed against cell surface antigens are generally more toxic to cells bearing the target antigen than immunotoxins prepared by coupling the ricin A chain to the antibody (IT-A) [3,4]. This suggests that the B chain of the toxin plays a role in the toxic process, perhaps by facilitating the translocation of the A

Abbreviations: CD, circular dichroism; FCS, fetal calf serum; GARIg, goat anti-rabbit Ig; hd, heat-denatured; Ig, immunoglobulin; IT-A, ricin A chain immunotoxin; IT-B, ricin B chain immunotoxin; PBS, phosphate-buffered saline; RAHIg, rabbit anti-human Ig; RIA, radioimmunoassay; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SPDP, *N*-succinimidyl-3-(2-pyridyldithio)propionate.

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chain from the plasma membrane to its site of action in the cytosol. Several studies have demonstrated that free ricin B chain can enhance the cytotoxicity of IT-As both in vitro [5,6] and in vivo [7]. The specific cytotoxicity of an IT-A can also be potentiated by the B chain linked to an antibody (IT-Bs) recognizing either the same cell surface determinant(s) [8] or the immunoglobulin (Ig) portion of the cell-bound IT-A [9]. It was recently shown that ricin B chain coupled to goat anti-rabbit Ig (GARIg-B) retained its ability to potentiate the toxicity of rabbit anti-human Ig coupled to A chain (RAHIg-A) to Daudi lymphoblastoid cells in vitro after chemical modification of the B chain with chloramine T [10]. Such treatment eliminated the galactose-binding capacity of the B chain and its ability to associate non-covalently with the A chain and suggested that neither of these properties of the B chain is essential for potentiation of toxicity in this model system.

In the present study, we demonstrate that native ricin B chain incubated at 37°C (heat-denatured (hd) B chain) in the absence of lactose undergoes a limited conformational change accompanied by the loss of its galactose-binding ability. As a result of this denaturation, the two oligomannose chains of the B chain, the intrachain disulfide bonds, and other previously inaccessible regions of the B chain become exposed. An IT-B prepared by coupling heat-denatured B chain to GARIg (GARIg-hdB), had no detectable galactose-binding ability. However, when GARIg-hdB was used in conjunction with RAHIg-A to treat Daudi cells, it potentiated the cytotoxicity of the IT-A. Taken together, these results indicate that, in this system, galactose-binding by the IT-hdB is not required for potentiation of the toxicity of an IT-A and suggests the possibility that unfolding of the B chain at the cell surface or intracellularly may lead to the expression of potentiating ability.

Materials and Methods

Heat denaturation of B chain. Ricin B chain used for heat denaturation experiments was isolated from ricin obtained from African castor beans (Croda Premier Oils, Hull, U.K.) as described previously [11,12] and was dialyzed into 50 mM borate buffer titrated to pH 9.0 with NaOH or into PBS (14 mM phosphate, 0.14 M NaCl, pH 7.2).

Solutions of B chain at 1 mg/ml were incubated at 28°C or 37°C and samples were removed at various times to ascertain the effect of heat on the saccharide-binding capacity of the lectin. This was measured by determining the proportion of the B chain (assessed by the absorbance at 280 nm) which retained affinity for a column of acid-treated Sepharose-4B (a galactose-based adsorbent) equilibrated with PBS at room temperature (21°C). Bound protein was eluted from the column with 0.1 M galactose/PBS.

Circular dichroism. CD Spectra of B chain solutions (at 1 mg/ml) were measured at ambient temperature with a JASCO J40CS instrument. CD measurements at 37°C were performed using a heated cell holder monitored by a thermal probe. Differential absorption (Δ) values were calculated on the basis of the mean residue molecular weight.

Analytical procedures. Isoelectric focusing was performed on a Pharmacia Pharm-Phast system with a Phast Gel IEF pH 3–9 gel used according to the manufacturer's protocol. The gel was stained with Coomassie blue R350. p*I* values were estimated by comparison with calibration proteins of known p*I* obtained from Pharmacia, Milton Keynes, U.K.

Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on 15% polyacrylamide gels using the discontinuous Tris-glycine system of Laemmli [13]. Protein bands were visualized by Coomassie blue staining. Apparent M_r values were estimated by comparison with molecular weight markers (Pharmacia).

Preparation of immunotoxins. Ricin A and B chains prepared from Vector Ricin (Burlingame, Vermont) were purified by ion-exchange and affinity chromatography as described by Fulton et al. [12]. Ricin A chain was coupled to affinity-purified rabbit anti-human Ig (RAHIg) using the *N*-succinimidyl-3-(2-pyridyldithio)propionate (SPDP) reagent as described previously [9].

IT-B and IT-hdB were prepared as described previously [10]. Native B chain (at 1.46 mg/ml) in 50 mM borate buffer (pH 9.0) was sterilized by passage through a 0.22 μm filter (Millex GV, Millipore), incubated for 48 h at 37°C and then

dialyzed into PBS. The resulting heat-denatured B chain preparation was passed over a column of Sepharose-asialofetuin (10.4 mg asialofetuin/ml gel) equilibrated in PBS to remove any galactose-binding material and the material not adhering to the column was filter-sterilized (overall yield, 62%). A control sample of native B chain was incubated in pH 9.0 buffer for 48 h at 4°C and then dialyzed into PBS. The two preparations of B chain were then each coupled to affinity-purified GARIg as described [10]. 0.1 M galactose was added to all buffer solutions used in the preparation and purification of the IT-B. The IT-B was separated from unconjugated B chain by gel filtration at 25°C on a Sephacryl S-200 column equilibrated with PBS containing 0.1 M galactose. Material with an apparent M_r of 150000–250000 was pooled and subjected to further purification by affinity chromatography on Sepharose-rabbit IgG in PBS/0.1 M galactose [10].

IT-B preparations were characterized by radioimmunoassay (RIA) and SDS-PAGE. GARIg-B contained an average of two to three B chain molecules per molecule of antibody. GARIg-hdB contained an average of one to two heat-denatured B chain molecules per molecule of antibody. Both preparations contained less than 1% unconjugated B chain (or heat-denatured B chain) as determined by SDS-PAGE and silver staining [10].

Cytotoxicity experiments. Daudi cells were maintained and treated as described previously [10]. Briefly, 10^5 cells were distributed into wells of a 96-well microtiter plate. In the case of free native or heat-denatured B chain, cells were cultured in the continuous presence of the B chain for 22 h at 37°C in a 5% CO_2 incubator in 200 µl of RPMI-1640 medium lacking leucine and containing 10% fetal calf serum (FCS) either in the presence or absence of 20 mM NH_4Cl. The cells were then pulsed with 1 µCi/well of [^{3}H]leucine (120 Ci/mmol) (New England Nuclear, Boston, MA) and the radioactivity incorporated into protein was measured 4–6 h later.

Cells were treated with immunotoxins as follows: different concentrations of RAHIg-A were added to triplicate wells containing 10^5 Daudi cells for 30 min at 4°C. The plates were centrifuged and the cells then washed three times in PBS. A fixed concentration (10^{-9} M) of GARIg-B or GARIg-hdB was then added for 30 min at 4°C. The plates were centrifuged and washed. The remainder of the assay was carried out in the presence of 20 mM NH_4Cl as described above. The cytotoxic effect of immunotoxin treatment was measured by comparing the [^{3}H]leucine incorporation of treated cells with that of untreated cells.

Binding of B chain to asialofetuin. Wells of a 96-well microtiter plate were treated with 0.1 ml of a 0.2 mg/ml solution of asialofetuin in PBS for 16 h at 4°C. Wells were washed five times with H_2O. Dilutions of GARIg-B, GARIg-hdB or GARIg were added to the wells in 100 µl/PBS at 25°C for 4 h. Wells were washed five times with H_2O. 10^5 cpm of ^{125}I-rabbit anti-goat Ig in 5% FCS were added to each well for 16 h at 4°C. Wells were washed five times with H_2O, cut out and the radioactivity was determined on a gamma counter. All samples were run in triplicate.

Results

Thermal inactivation of ricin B chain

When native ricin B chain was incubated at 37°C in 50 mM borate buffer (pH 9.0) in the absence of lactose, it progressively lost its capacity to bind to a column of Sepharose-4B (Fig. 1). After 3 h of incubation, approx. half of the B chain failed to bind. The inactivation was about 4-fold faster in 12.5 mM as compared to 50 mM borate buffer showing that the thermal stability of the B chain was reduced at lower ionic strength. When the B chain was incubated at 37°C in PBS (pH 7.2), its Sepharose-binding ability was lost at the same rate as in 50 mM borate buffer (pH 9.0) but at the lower pH value, all the heat-denatured B chain precipitated. Little (less than 5%) inactivation of binding occurred if the B chain was incubated at pH 7.0 or pH 9.0 in the presence of 0.2 M lactose at 37°C or in the absence of lactose at 28°C. In contrast with the isolated B chain, the Sepharose-binding ability of intact ricin was unaffected by prolonged incubation at 37°C in the absence of lactose.

B chain that had been reacted with iodoacetamide (to *S*-amidocarboxymethylate the single free thiol group) denatured at the same rate as unmodified preparations of the B chain. Similarly, the

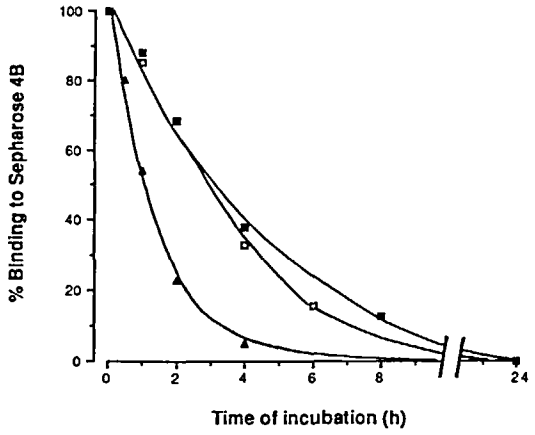

Fig. 1. Thermolability of the Sepharose-binding ability of ricin B chain. B chain was incubated at 37 °C in PBS (pH 7.2) (□), in 50 mM borate buffer (pH 9.0) (■) or in 12.5 mM borate buffer (pH 9.0). (▲). The inactivated B chain precipitated at pH 7.2 but remained soluble at pH 9.0.

inclusion of 10 mM 2-mercaptoethanol or 10 mM dithiothreitol in the incubation mixture did not affect the denaturation rate showing that thiol–disulfide exchange within the protein was not responsible for denaturation. Both native and heat-denatured B chain gave a major band on isoelectric focusing with a p*I* of 4.05, calculated relative to standard proteins of known p*I*, indicating that no detectable chemical change such as deamidation had occurred during the incubation. Analyses by SDS-PAGE (see below) gave no indication of proteolytic cleavage of the B chain after incubation at 37 °C for 48 h in the absence of lactose excluding the possibility that loss of galactose-binding ability was caused by thermal activation of a contaminating proteinase. These findings strongly suggested that ablation of galactose-binding was due to a heat-induced conformational change in the B chain.

Conformational changes accompanying thermal denaturation of ricin B chain

The CD spectrum of heat-denatured B chain at pH 9.0 differed from that of the native B chain at the same pH (Fig. 2). The negative band at 283 nm attributable to tryptophan was completely absent and is consistent with the interpretation that a significant alteration in the tertiary structure of the B chain close to the tryptophan chromophore(s) and probably involving the saccharide-binding sites had occurred [24]. The CD bands attributable to disulfide bonds in constrained conformations, i.e., at 232 nm and at 300–325 nm [24], were also absent and a new weak positive band at 255 nm was evident in the spectrum of the heat-denatured B chain. Unconstrained disulfide bonds are known to produce CD bands centered at 255 nm [14]. These changes demonstrate a loss of ordered structure in the regions of the polypeptide chain linked by disulfide bonds. During the course of heat treatment, both the 232 nm and 283 nm CD bands decreased at the same rate at which the saccharide-binding ability was lost, indicating that changes in the vicinity of the tryptophan residue(s) in the galactose-binding site and of disulfide bonds occurred simultaneously. Thus, these change were responsible for the loss of galactose binding.

The 200–225 nm CD band in the B chain spectrum was fundamentally unchanged after heat denaturation implying that the overall secondary structure of the native B chain was preserved. Thus, the loss of saccharide-binding ability was apparently due to a change in the relative disposition of folded units of secondary structure. Importantly, the denatured B chain did not revert to its galactose-binding configuration when dialyzed into galactose-containing buffers.

Exposure of disulfide bonds in ricin B chain as a result of heat denaturation

SDS-PAGE under nonreducing conditions showed that the original preparation of native B chain and B chain which had been incubated for

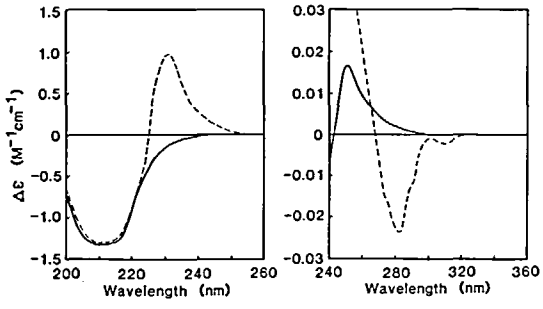

Fig. 2. CD spectra of native and heat-denatured B chains. B chain (— — —) and heat-denatured B chain (———) determined in 50 mM borate buffer (pH 9.0).

up to 48 h in 12.5 mM borate buffer (pH 9.0) contained a mixture of monomeric and dimeric molecules (Fig. 3). The dimers dissociated completely to monomers under reducing conditions (data not shown) indicating that they were disulfide-bonded. After 24 h and 48 h of incubation, the intensity of the monomer and dimer bands was decreased and material staining with Coomassie blue was evident at the top of both the stacking and resolving gels indicating the formation of high-molecular-weight polymers. This high-molecular-weight material dissociated into monomers under reducing conditions indicating that it resulted from cross-linking due to thiol–disulfide exchange. The increased tendency of the disulfide bonds in the B chain to participate in disulfide exchange reactions was apparently due to the conformational change induced by incubation at 37° C because no cross-linking occurred when the B chain was incubated at the same temperature in the presence of 0.2 M lactose or at 28° C in the absence of lactose.

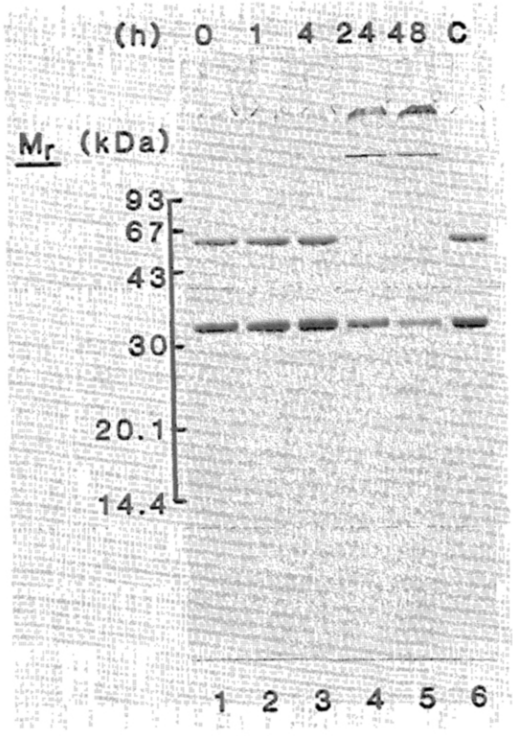

Fig. 3. SDS-PAGE of B chain incubated at 37° C. Samples were electrophoresed under nonreducing conditions. The band at 32 kDa is monomeric B chain and at 64 kDa is dimeric B chain. Lanes 1–5, B chain incubated at 37° C for 0, 1, 4, 24 and 48 h. Lane 6, B chain (control) kept at 28° C for 48 h.

It is important to note that appreciable disulfide-mediated polymerization only occurred after 4 h, at a time when virtually all the B chain had undergone heat-induced denaturation (Fig. 1). This suggests that the conformational change induced by heat exposes disulfide bonds in the B chain that are normally hidden from the solvent within the protein structure and this then allows cross-linking to take place. This interpretation is consistent with the evidence from CD studies described above that a change in the conformation of the disulfide bonds in the B chain during heating occurs simultaneously with unfolding of the regions of the molecule involved in galactose binding. Furthermore, attempts to reduce the disulfide bonds of native ricin B chain with reducing agents before or during incubation at 37° C did not accelerate the rate at which the galactose-binding ability was lost.

Cytotoxicity of heat-denatured B chain to Daudi cells in vitro

In cytotoxicity experiments with Daudi cells in vitro, free native B chain inhibited the incorporation of [^{3}H]leucine by 50% relative to untreated cells at a concentration (IC_{50}) of $6 \cdot 10^{-8}$ M. This toxicity apparently depends upon the ability of the B chain to bind to the cells by means of its galactose-binding sites because the A chain content of the B chain was less than 1 in 10^8 molecules [10]. In contrast, the IC_{50} value for the toxicity of free heat-denatured B chain to Daudi cells was more than $3 \cdot 10^{-7}$ M. When 20 mM NH_4Cl was included in the incubation mixture, the ability of native B chain to inhibit protein synthesis in Daudi cells was enhanced ($IC_{50} = 2 \cdot 10^{-9}$ M), whereas there was no significant increase in the cytotoxicity of the heat-denatured B chain ($IC_{50} > 3 \cdot 10^{-7}$ M). The inability of heat-denatured B chain to inhibit protein synthesis in Daudi cells in vitro is consistent with its inability to bind to cell surface saccharides.

Potentiation of the cytotoxic activity of RAHIg-A on Daudi cells by GARIg-hdB

The saccharide-binding ability of IT-Bs prepared with native or heat-denatured B chain was

determined by measuring their binding to asialofetuin in an RIA. As shown in Fig. 4, GARIg-B retained asialofetuin binding ability, but GARIg-hdB bound to the asialofetuin-coated plate at levels similar to that of GARIg alone. This result indicates that the heat-denatured B chain did not regain its ability to bind to a galactose-containing glycoprotein following conjugation to antibody. The potentiating ability of GARIg-B was tested by adding it at a single nontoxic concentration (10^{-9} M) to Daudi cells which had been pretreated with RAHIg-A at different concentrations. In three separate experiments, GARIg-B enhanced the cytotoxicity of the IT-A in cells cultured with NH_4Cl by 8- to 11-fold. The mean IC_{50} for RAHIg-A treatment alone, $1.2 \cdot 10^{-9}$ M, was reduced to $1.4 \cdot 10^{-10}$ M by piggyback treatment with GARIg-B (Fig. 5). GARIg-hdB also enhanced the toxicity of RAHIg-A to Daudi cells in three separate experiments by about 3- to 5-fold. The mean IC_{50} for the combined treatment with

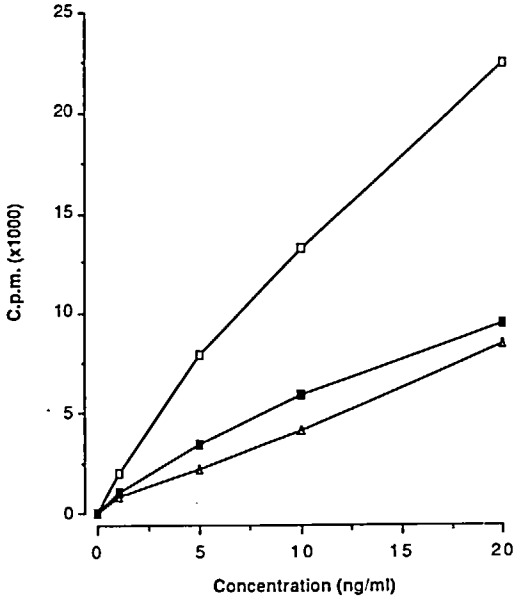

Fig. 4. The ability of antibody or IT-Bs to bind to asialofetuin. Individual wells of asialofetuin-coated microtiter plates were treated with GARIg-B (□), GARIg-hdB (△) or unconjugated GARIg (■) at different concentrations (ng/ml), washed and the bound material was detected using a second layer of ^{125}I-rabbit anti-goat Ig (cpm) as described in the Materials and Methods.

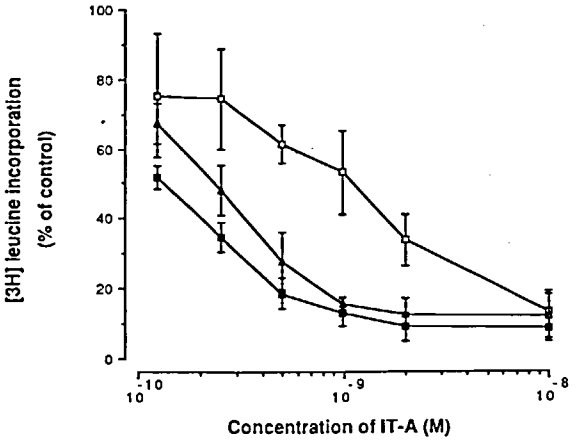

Fig. 5. Potentiation of RAHIg-A by GARIg-hdB. Daudi cells were treated with various concentrations of RAHIg-A and then with 10^{-9} M GARIg-B (■) or GARIg-hdB (▲), or medium only (□). Each point represents the mean value of at least three experiments and the error bars show the standard deviations.

RAHIg-A and GARIg-hdB was $2.3 \cdot 10^{-10}$ M. Therefore, after heat denaturation and conjugation to GARIg, the B chain (as a GARIg-hdB) which had lost all detectable galactose-binding ability, retained 60% of its potentiating activity as compared to GARIg-B. The decrease in potentiating activity of GARIg-hdB as compared to GARIg-B may be due to the fact that the GARIg-B contained two or three B chains per antibody molecule while the GARIg-hdB contained one or two B chains per antibody.

As demonstrated previously [10], unconjugated GARIg or GARIg conjugated to ovalbumin were unable to potentiate RAHIg-A cytotoxicity. As noted above, free heat-denatured B chain, in contrast to native B chain, did not enhance the killing of Daudi cells treated with RAHIg-A. Therefore, the potentiating ability of the heat-denatured B chain was only observed when it was delivered to cell-bound RAHIg-A by means of conjugation to GARIg.

Discussion

The present experiments demonstrate that highly purified ricin B chain rapidly loses saccharide-binding ability when incubated at 37°C in the absence of lactose. The rate of denaturation

of the B chain correlates with the rate at which it undergoes a conformational change. Circular dichroism of the B chain before and during heat denaturation revealed that the environment of tryptophan chromophore(s) was altered and that the constrained conformation of disulfide bonds was relaxed during inactivation of galactose-binding sites. However, the overall secondary structure of the B chain was unaltered suggesting that the loss of galactose-binding ability was due to a limited unfolding of the native B chain. The presence of a saturating concentration of lactose prevented thermal denaturation. CD analyses have previously shown that lactose causes no major change in the overall conformation of the B chain [24]. Therefore, lactose probably strengthens the interactions between the different polypeptide loops of the B chain that form the galactose-binding sites and stabilizes the overall protein structure so as to prevent heat denaturation.

The limited conformational change that the B chain undergoes during heating has other consequences besides disrupting the structure of the galactose-binding sites. First, disulfide bonds that were shielded from the solvent by the compact folded structure of native B chain became exposed upon denaturation. This led to cross-linking of B chains on prolonged incubation at pH 9.0 through thiol–disulfide exchange. However, cross-linking only occurred after the B chain had lost all galactose-binding ability suggesting that the disulfide bonds were exposed as a result of the heat-induced conformational change. In this respect, CD analyses provided evidence that residues near the disulfide bonds in the molecule changed during denaturation. Second, the chitiobiosyl core sections of both the N-linked oligomannose chains of the B chain became susceptible to attack by endoglycosidases. It was previously found that lactose prevented the removal of one of these oligosaccharide chains by protein: *N*-glycosidase F at pH 9.0 and 37°C [15]. This protective effect is apparently due to the ability of lactose to inhibit heat-induced unfolding of the B chain.

The heat-denatured B chain and IT-hdB had no detectable asialofetuin binding ability, yet GARIg-hdB could potentiate the cytotoxic action of RAHIg-A on Daudi cells in a manner that was 60% as effective as that obtained with GARIg-B. This finding confirms and extends earlier findings that IT-Bs prepared with chloramine T-treated B chains which were unable to bind to asialofetuin were only 2- to 8-fold less effective at potentiating the cytotoxicity of RAHIg-A than GARIg-B [10]. We have subsequently determined the CD spectrum of chloramine T-treated B chain (data not shown) and found that it, too, lacks the 232 nm and 283 nm bands characteristic of the native structure of the B chain. These results indicate that this oxidation procedure causes unfolding of the polypeptide in a manner similar to that observed with heat denaturation.

The finding that potentiation of the toxicity of RAHIg-A to Daudi cells by IT-B is not dependent upon the saccharide-binding ability of the B chain is also consistent with the results of Thorpe et al. [16] who found that intact ricin immunotoxins prepared so that the galactose-binding sites of the toxin were sterically blocked by attachment to antibody were as toxic to target cells in vitro as ricin immunotoxins which retained galactose-binding ability. This contrasts with the results of Youle et al. [17] who found that a conjugate of mannose 6-phosphate coupled to ricin which had been chemically modified to attenuate its galactose-binding ability was less toxic to cultured fibroblasts than a conjugate prepared with unmodified ricin suggesting that, in some systems, the cytotoxic route of entry of the A chain depends on galactose-binding by the B chain.

The mechanism by which ricin B chain, denatured either by incubation at 37°C or by chloramine T oxidation and coupled to GARIg, is able to enhance the cytotoxic action of RAHIg-A against Daudi cells is unclear. Based on the present results, this function, unlike galactose binding, is largely retained upon denaturation. One hypothesis is that as a result of denaturation, the B chain disrupts the membrane structure of an intracellular vesicle and forms a pore that allows the A chain of the IT-A to pass into the cytosol. Although the insertion of the B chain into liposomal [18,19] and viral [20] membranes has been demonstrated, the evidence that this interaction is involved in mediating A chain translocation is still lacking. A second possibility is that the oligomannose chains of the B chain mediate potentiation, e.g., by binding GARIg-B to a cellular

saccharide-binding receptor and thereby diverting the RAHIg-A to an intracellular compartment favoring the translocation of A chain into the cytosol. There is evidence that the oligosaccharide chains of the B chain determine toxicity from the finding that GARIg coupled to chemically deglycosylated B chain, in which the terminal mannose residues had been destroyed, was 4- to 8-fold less effective at potentiating RAHIg-A toxicity to Daudi cells in vitro than an IT-B containing native B chain [21]. Similarly, ricin formed by recombining native A chain with α-mannosidase-treated B chain had 4-fold lower toxicity to cells than ricin reformed from native A and B chains [23]. Furthermore, swainsonine, an inhibitor of α-mannosidase II, potentiated the toxicity of ricin to rat bone marrow macrophages suggesting that, when the activity of a putative intracellular mannosidase was inhibited, the toxicity of ricin was increased [22]. It is unlikely, however, that mannose residues are the sole determinant of intracellular recognition, because GARIg linked to ovalbumin, a mannosylated protein, did not potentiate the toxicity of RAHIg-A to Daudi cells [10].

In conclusion, we have demonstrated that an IT-B containing conformationally altered ricin B chain lacking detectable galactose-binding ability retained the ability to potentiate the toxicity of an IT-A in an in vitro cytotoxicity assay. The susceptibility of the isolated B chain to such conformational changes indicates that expression of the potentiating activity of native B chain coupled to antibody depends upon denaturation of the B chain at the cell surface or within an intracellular compartment.

Acknowledgments

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