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Influence of the Membrane on T-2 Toxin Toxicity in *Saccharomyces* spp.

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In growing cells of *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis*, T-2 toxin inhibits cell growth. We have examined the role of the yeast membranes in the uptake mechanism(s) of T-2 toxin. The effects of membrane-modulating agents, ethanol, cetyltrimethylammonium bromide, Triton X-100, and heat were studied; these agents were found to increase the sensitivity of the yeasts toward T-2 toxin. In the presence of 5% (vol/vol) ethanol, 2 μg of T-2 toxin per ml caused complete inhibition of growth. In the presence of 1 μg of cetyltrimethylammonium bromide per ml, yeast cells became sensitive to T-2 toxin, starting with a concentration of 0.5 μg/ml. Triton X-100 at concentrations below 1% (vol/vol) sensitized the cells toward T-2 toxin, but at higher concentrations it protected the cells from T-2 toxin. Temperatures of incubation between 7 and 30°C influenced the growth reduction caused by T-2 toxin. The greatest observed reduction of growth in T-2 toxin-treated cultures occurred at 30°C. To further prove that the membrane influences the interaction of T-2 toxin with yeasts, we have studied a yeast mutant with a reduced plasma membrane permeability (G. H. Rank et al., Mol. Gen. Genet. **152**:13–18, 1977). This yeast mutant proved to be resistant to T-2 toxin concentrations of up to 50 μg/ml. These results show that the membrane plays a significant role in the interaction of T-2 toxin with yeast cells.

T-2 toxin is a member of the trichothecene family of mycotoxins. The trichothecenes are produced by a variety of fungi found worldwide. Trichothecene-producing fungi have been isolated from moldy grain found in Canada and the United States (4, 5, 13, 19).

T-2 toxin has no effect against the growth of bacteria (2), yet its action on eucaryotic cells is exceedingly complex, and no clear-cut model has been proposed to explain its mode of action. Ueno and co-workers (21) have found that T-2 toxin inhibits protein synthesis in rabbit reticulocytes. Linnainmaa and co-workers (12) have shown that T-2 toxin has an action similar to that of colchicine, that is, T-2 toxin is a mitotic inhibitor. T-2 toxin has been shown to break the chromosome of rat lymphocytes (11) but not to be mutagenic or to affect mitotic recombination (10, 20).

We have recently reported on the growth inhibitory action of T-2 toxin on *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis* (18). Inhibition was dependent on the carbon source, concentration of T-2 toxin, and length of exposure to T-2 toxin. In addition, the mitochondrial action of the toxin was detected, as the growth inhibition on glycerol was considerably greater than on glucose.

In this report, we further describe the interaction of T-2 toxin with yeasts and demonstrate the dependence of T-2 sensitivity on membrane integrity and function.

(A preliminary report of this work was presented at the Annual Meeting of the Canadian Society for Microbiology, Winnipeg, Manitoba, 19 to 23 June 1983 [K. T. Schappert and G. G. Khachatourians].)

MATERIALS AND METHODS

A previous publication (18) provides details of yeast growth and T-2 toxin assay, using *S. carlsbergensis* L-5. *S. cerevisiae* GR-359 was supplied by G. Rank, University of Saskatchewan, Saskatoon, Canada, and has been characterized for its genetic and biochemical properties in several publications (notably in references 15 and 16). *S. cerevisiae* GR-359 is a haploid yeast strain with the genotype **a** *his6 met trp1 oli* PR1-1 (*ery*^{r} *rho*$^+$ *cir*$^+$) and contains a mutation altering its membrane permeability. The source for T-2 toxin was Myco-lab Co., Chesterfield, Mo. The toxin had a purity of 99%, as supplied by the producer. Cetyltrimethylammonium bromide (CTAB) and Triton X-100 were purchased from Sigma Chemical Co., St. Louis, Mo. The growth medium used for the culturing of yeasts was 10 g of yeast extract, 10 g of peptone broth, and 20 g of glucose per liter of distilled water. Optical density of the cultures was determined by using a Horizon spectrophotometer at 610 nm (model 5965-50; Horizon Ecology Co., Chicago, Ill.).

RESULTS

For T-2 toxin (Fig. 1) to exert its toxic effects on a cell, it must first interact with the plasma membrane of the cell. Once the toxin has penetrated the cell, it would then interact with potential intracellular targets. Therefore, the biological action of T-2 toxin is dependent on membrane function and integrity.

Effects of membrane-modulating agents. The ability of CTAB (a membrane-modulating cationic detergent), ethanol, and Triton X-100 (a nonionic detergent) to potentiate the growth-retarding effects of T-2 toxin on *S. carlsbergensis* was tested.

CTAB at concentrations of ≥1 μg/ml reduced growth of *S. carlsbergensis* in the absence of T-2 toxin (Fig. 2). At concentrations of ≥20 μg/ml, complete cessation of growth occurred.

Figure 2 illustrates the effect of T-2 toxin at 10 μg/ml on the growth reduction caused by CTAB. In the presence of CTAB at 5 μg/ml, a concentration which alone caused only 20% reduction in growth, T-2 toxin causes complete growth inhibition. When the cells were incubated with CTAB at 1 μg/ml, a further 20% reduction in T-2 toxin-induced growth inhibition occurred.

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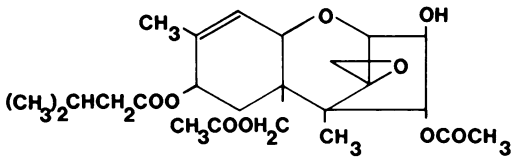

FIG. 1. Chemical structure of T-2 toxin.

The response of yeast cells to various concentrations of T-2 toxin in the presence of CTAB at 1 μg/ml was also studied. T-2 toxin at less than 1 μg/ml caused no measurable effect on growth (Fig. 3). In the presence of 1 μg of CTAB per ml, yeast cells became sensitive to the growth retardation effects of T-2 toxin, starting with a concentration of 0.5 μg/ml.

It has been reported that ethanol has a modulating effect on biological membranes (6, 8). We tested this in our system. Ethanol at levels from 0.5 to 5% (vol/vol) increased the amount of growth reduction caused by 10 μg of T-2 toxin per ml (data not shown). Since ethanol at 5% (vol/vol) caused the greatest increase in sensitivity of yeast cells to the toxin, the toxin-potentiating effects of ethanol were tested at this concentration (Fig. 3). Ethanol greatly potentiated the growth retardation caused by T-2 toxin. In the presence of ethanol, growth of *S. carlsbergensis* was completely inhibited at 2 μg of T-2 toxin per ml. Yeast cells also became sensitive to T-2 toxin concentrations that were nontoxic in the absence of ethanol. In addition, ethanol at 5% (vol/vol) increased the sharpness of the toxin sensitivity profile of *S. carlsbergensis*. This was unlike CTAB which shifted the sensitivity of *S. carlsbergensis* without changing the overall shape of the curve. In the presence of 5% (vol/vol) ethanol, the noncytotoxic level of T-2 toxin was 0.2 μg/ml.

The nonionic detergent Triton X-100 produced a curious effect on the growth retardation caused by T-2 toxin (Fig. 4). At concentrations below 1% (vol/vol), Triton X-100 sensitized the cells towards T-2 toxin. However, at concentrations greater than 1% (vol/vol), it protected the cells from T-2 toxin. Triton X-100 concentrations greater than 5% (vol/vol) proved toxic to the cells.

Effect of elevated temperatures. It is well known that temperature affects biological processes in cells (7). For example, changes in temperature can alter growth kinetics and membrane fluidity. Elevated temperatures can also denature proteins and affect membrane integrity (22).

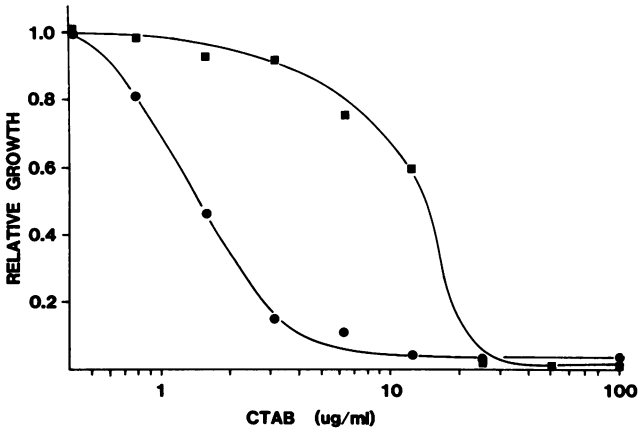

FIG. 2. Effect of CTAB on growth of *S. carlsbergensis* in the absence of T-2 toxin (■) and in the presence of 10 μg of T-2 toxin per ml (●). Note that a relative growth value of 1.0 represents 50% reduction of growth with T-2 toxin at 10 μg/ml.

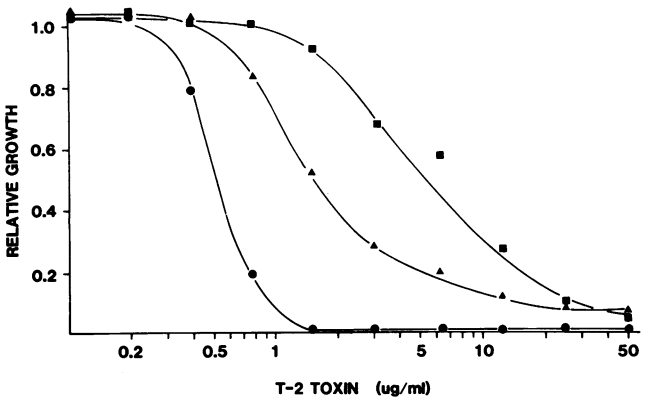

FIG. 3. Effect of CTAB and ethanol on growth reduction caused by T-2 toxin. Growth inhibition caused by T-2 toxin alone (■), in the presence of 1 μg of CTAB per ml (▲), and in the presence of ethanol (5% [vol/vol]) (●).

Table 1 shows the effect of temperature on growth rate reduction studied in *S. carlsbergensis*. There was a less than 3% reduction in specific growth rates for T-2-treated cultures incubated at 7 and 15°C. At temperatures of 20, 25, and 30°C, there was 9, 34, and 66% reduction, respectively, in the specific growth rates for toxin-treated cultures. As the growth temperature is increased, *S. carlsbergensis* becomes increasingly sensitive to the growth-retarding action of T-2 toxin (Fig. 5). Based on data presented (Table 1), the effect of T-2 toxin was maximal when yeast cells were cultured at 30°C. It was observed that T-2 toxin did not have growth retardation effects below 15°C. In addition, a heat pulse (56°C for 5 min) increased the sensitivity of *S. carlsbergensis* to T-2 toxin by fivefold.

T-2 toxin sensitivity of *S. cerevisiae* mutant with altered membrane. To correlate the effect(s) of membrane-modulat-

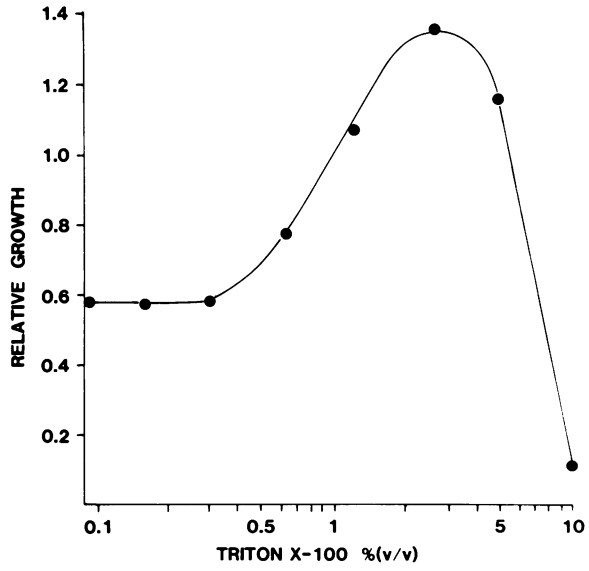

FIG. 4. Influence of Triton X-100 on growth reduction caused by 10 μg of T-2 toxin per ml.

TABLE 1. Effect of temperature on growth rate inhibition caused by T-2 toxin

Temp (°C)	Specific growth rate (h^{-1})[a]	
	Control	T-2 toxin treated[b]
7	0.0122	0.0123
15	0.1311	0.1285
20	0.1507	0.1380
25	0.1660	0.1103
30	0.2211	0.0770

[a] Results are the averages of duplicates.
[b] Cells were treated with 10 μg of T-2 toxin per ml.

ing agents on T-2 toxin sensitivity of yeast cells, we next examined a number of *S. cerevisiae* mutants with defined membrane lesions (15–17). Of these strains, strain GR-359 provided the most interesting results. This particular single-gene mutant of *S. cerevisiae* processes multiple resistance to many inhibitors of growth, yet confers increased sensitivity to other inhibitors (15).

Figure 6 illustrates the effects of CTAB and T-2 toxin on the growth of *S. cerevisiae* GR-359. GR-359 was resistant to T-2 toxin at concentrations of up to 25 μg/ml, and 50 μg/ml caused only a marginal growth retardation. *S. cerevisiae* possessing the wild-type gene to *oli* PR1-1 were sensitive to the growth-retarding effects of T-2 toxin (data not shown).

S. cerevisiae GR-359 was sensitive to the growth retardation caused by CTAB. It is interesting that at CTAB concentrations of less than 20 μg/ml, GR-359 showed similar growth reduction kinetics to those of *S. carlsbergensis* (Fig. 2 and 3). Both yeasts were resistant to CTAB at 1 μg/ml, yet they were only capable of ca. 60% growth when CTAB was present at 10 μg/ml. This suggests that CTAB acts similarly in both yeasts. However, when cultured in the presence of CTAB and T-2 toxin, *S. cerevisiae* GR-359 exhibited growth reduction similar to that caused by CTAB alone. That is, CTAB did not sensitize strain GR-359 to T-2 toxin as it did wild-type *S. carlsbergensis*.

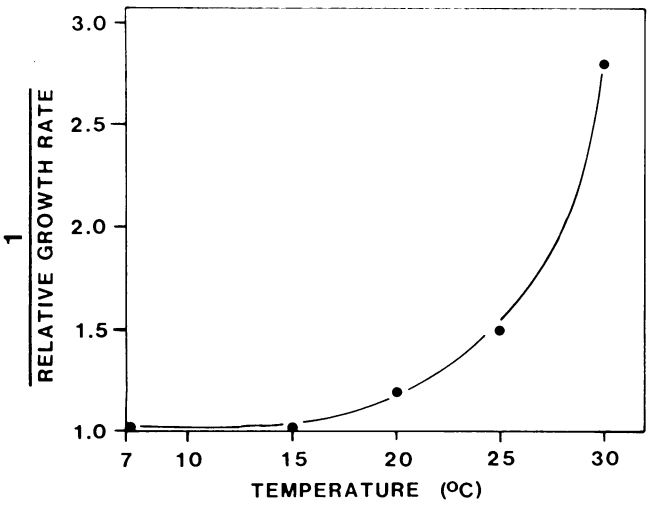

FIG. 5. Influence of incubation temperature on growth rate reduction caused by T-2 toxin at 10 μg/ml. The *x* axis indicates growth temperature. The *y* axis shows the reciprocal value for relative growth rate, defined as the ratio of growth rates for T-2-treated and untreated cultures.

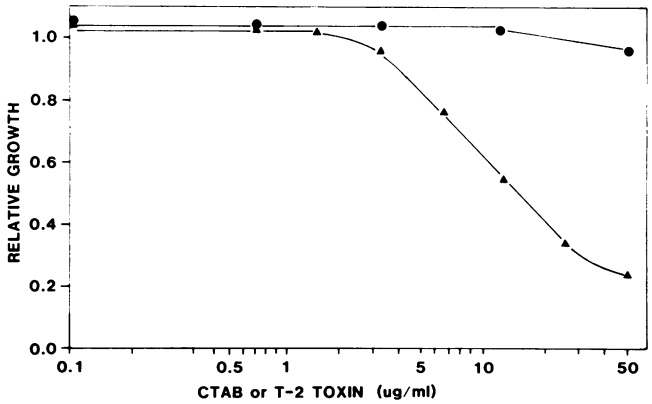

FIG. 6. Effect of CTAB and T-2 toxin on growth of *S. cerevisiae* GR-359. Cells were incubated either with CTAB (▲) or T-2 toxin (●) at concentrations indicated on the *x* axis.

DISCUSSION

In this paper, we have described the effects of membrane-modulating agents on the growth reduction caused by T-2 toxin in *S. carlsbergensis* and a membrane mutant of *S. cerevisiae*. All agents tested magnified the growth-reducing effects of T-2 toxin in *S. carlsbergensis*.

In a previous report (18), we showed that T-2 toxin at 10 μg/ml caused a ca. 50% reduction in growth. This concentration of T-2 toxin was used to determine whether CTAB would alter the sensitivity of yeasts toward T-2 toxin. Treatment of yeasts with CTAB at 5 μg/ml resulted in only a 15% inhibition of growth. However, when combined with 10 μg of T-2 toxin per ml, CTAB completely inhibited growth of yeast cells. In this regard, the cumulative effect is greater than the sum of the individual effects of CTAB and T-2 toxin. Triton X-100 produced a curious effect on the growth reduction caused by T-2 toxin in *S. carlsbergensis*. At concentrations greater than 1% (vol/vol), Triton X-100 protected the cells from T-2 toxin, yet at lower concentrations it enhanced the sensitivity of *S. carlsbergensis*. The protection offered by Triton X-100 may be due to its ability to dissociate hydrophobic interactions. Norman and co-workers (14) have shown that Triton X-100 dissociated the hydrophobic interaction between cholesterol and the polyene antibiotic filipin. We have recently shown by using charge-shift electrophoresis that T-2 toxin is a hydrophobic molecule (M. I. C. Gyongyossy-Issa, E. J. Christie, and G. G. Khachatourians, Appl. Environ. Microbiol., in press). Thus it is conceivable that a nonionic detergent could bind the hydrophobic T-2 toxin and prevent it from acting.

Ethanol, whose action on the membrane has been well studied, was shown to potentiate the action of T-2 toxin in *S. carlsbergensis*. The yeast species used in this study was a brewing yeast and was thus tolerant to ethanol concentrations of up to 16% (vol/vol) (3). Ethanol at 5% (vol/vol) greatly potentiated the growth inhibitory action of T-2 toxin. In the absence of ethanol, 2 μg of T-2 toxin per ml caused minor reduction in the growth of this yeast species. In the presence of ethanol (5% [vol/vol]), 2 μg of T-2 toxin per ml caused complete cessation of growth.

The effect of growth temperature on the fluidity of microbial membranes is well known (22). Lower temperatures decrease fluidity, whereas higher temperatures increase fluidity. Still higher temperatures cause damage to components of membranes. We have shown that the temperature of

incubation influences the toxicity seen in *S. carlsbergensis*. Temperatures below 15°C drastically decreased the toxicity induced by T-2 toxin. The action of T-2 toxin increased above 15°C and was maximal at 30°C. Above this temperature, the yeasts were not capable of growth. At lower temperatures, T-2 toxin would probably have difficulty in penetrating the cell or interacting with components of the membrane. At higher temperatures, the effect would be reversed, thus allowing for a greater interaction between T-2 toxin and the membrane. Beuchat (1) has shown that heat-treated *S. cerevisiae* is unable to withstand low water activity and high osmotic pressures and has increased sensitivity to surface active agents. In addition, Ingram (9) has shown the synergistic action between various chaotropic agents on the growth of cells.

The exact nature of the interaction of CTAB, Triton X-100, ethanol, and heat with T-2 toxin sensitivity is not at present known. We have shown that each of these potentiates the action of T-2 toxin. This suggests that the interaction is more complex than the simple combined action of the two agents separately. It may well be that T-2 toxin is affecting the membrane such that with the added stress of another membrane-perturbing agent, lethality to the cells results. Support for the above hypothesis comes from the work presented here as well as from the observation (M. I. C. Gyongyossy-Issa and G. G. Khachatourians, Biochim. Biophys. Acta, in press) that T-2 toxin is hydrophobic and has a natural affinity for murine lymphocyte membranes. This affinity would extend to all membranes in a cell, including cytoplasmic, nuclear, and mitochondrial and the endoplasmic reticulum. Disruption of membrane function would therefore have many effects on a cell.

The work presented here indicates that the toxic action of T-2 toxin is dependent on membrane integrity. Certainly, more work is necessary to prove this fact. Membrane-modulating agents enhanced the toxic action in wild-type yeasts but did not do so in a membrane mutant of *S. cerevisiae*. *S. cerevisiae* GR-359 proved to be resistant to the growth inhibitory action of T-2 toxin at concentrations of up to 50 μg/ml. This suggests that the membrane is important in T-2 toxin sensitivity.

The measurement of toxicity in our experiments to date has been the amount of growth occurring in the presence of T-2 toxin. We are currently investigating the effects of T-2 toxin on other cellular processes in yeasts and mechanisms of resistance in several yeast mutants that we have recently isolated. We hope to elucidate the mechanism of action and to identify the intracellular targets of T-2 toxin in yeast cells.

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