INFLUENCE OF THE MEMBRANE ON HT-2 TOXIN TOXICITY IN *SACCHAROMYCES CARLSBERGENSIS*

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SUMMARY

In growing cells of *Saccharomyces carlsbergensis* HT-2 toxin inhibits cell growth. We have examined the role of the yeast membranes in the uptake mechanisms of HT-2 toxin. The effects of membrane-modulating agents, ethanol, cetyltrimethylammonium bromide, Triton, and heat were studied, these agents were found to increase the sensitivity of the yeasts toward HT-2 toxin. In the presence of 1 _g of cetyltrimethylammonium bromide per ml, yeast cells became sensitive to HT-2 toxin, starting with a concentration of 0,5 _g/ml. Triton at concentrations below 1 % (vol/vol) sensitized the cells toward HT-2 toxin, but a higher concentrations it protected the cells from HT-2 toxin. Temperatures of incubation between 7 and 30 °C influenced the growth reduction caused by HT-2 toxin. The greatest observed reduction of growth in HT-2 toxin-treated cultures occurred at 30 °C.

INTRODUCTION

HT-2 toxin 15-Acetoxy-8-(3-methylbutanoyl)-12,13-epoxy-trichotec-9-ene-3 , 4 , 8a, 15-tetrol, is a member of the trichotechene family of micotoxins. HT-2 toxin is produced by *Fusarium culmorum, F. poae, F. solani, F. sporotrichiella, F. sporotrichioides* and *F. tricinctum*. These fungi are known to contaminate agricultural products worldwide. The effect of HT-2 toxin on the growth and metabolism of yeasts has only been investigated to a very limited extent (Burmeister, 1970). The effects of this toxin on yeast physiology and its mechanism of action have not been detailed. In the present paper we describe the results of our studies on the interaction of HT-2 toxin with yeast.

MATERIALS AND METHODS

Yeast strains. *Saccharomyces carlsbergensis* came from the culture collection of A. Halász, of this department.
Media. All experiments were conducted in yeast extract-peptone-glucose medium. This medium consisted of (per 1000 ml) 2 g of yeast extract, 2 g of peptone and 20 g of glucose.
Optical density measurements. The optical density of the cultures was measured at 610 nm with a Philips spectrophotometer.

RESULTS AND DISCUSSION

Effects of membrane-modulating agents. For HT-2 toxin to exert its toxin effects a cell, it must firs interact with the plasma membrane of the cell. Once the toxin has penetrated the cell, it would then interact with potential intracellular targets. The ability of CTAB (a membrane-modulating cationic detergent), ethanol, and Triton (a nonionic detergent) to potentiate the growth-retarding effects of HT-2 toxin on *S. carlsbergensis* was tested. CTAB at concentrations of 1 _g/ml reduced growth of *S. carlsbergensis* in the absence of HT-2 toxin (Fig.1) At concentrations of 20 _g/ml complete cessation of growth occurred. Fig. 1 illustrates the effect of HT-2 toxin at 10 _g/ml on the growth reduction caused by CTAB. In the presence of CTAB at 5 _g/ml, a concentration which alone caused only 20 % reduction in growth, HT-2 toxin causes complete growth inhibition. When the cells were incubated with CTAB at 1 _g/ml, a further 20 % reduction in HT-2 toxin-induced growth inhibition occurred. The response of yeast cells to various concentrations of HT-2 toxin in the presence of CTAB at 1 _g/ml was also studied. HT-2 toxin at less than 1 _g/ml caused no measurable effect on

growth (Fig.2). In the presence of 1 _g of CTAB per ml, yeast cells became sensitive to the growth retardation effects of HT-2 toxin, starting with a concentration of 0,5 _g/ml. It has been reported that ethanol has a modulating effect on biological membranes (Ingram, L.O. 1976). 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 _g of HT-2 toxin per ml. 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. 2). Ethanol greatly potentiated the growth retardation caused by HT-2 toxin. In the presence of ethanol, growth of *S. carlsbergensis* was completely inhibited at 2 _g of HT-2 toxin per ml. Yeast cells also became sensitive to HT-2 toxin concentrations that were non-toxic 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 non-cytotoxic level of HT-2 toxin was 0,2 _g/ml. The nonionic detergent Triton produced a curious effect on the growth retardation caused by HT-2 toxin (Fig. 3). At concentration below 1 % (vol/vol) , Triton sensitized the cells towards HT-2 toxin, however, at concentrations greater than 1 % (vol/vol) it protected the cells from HT-2 toxin. Triton 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. For example, changes in temperature can alter growth kinetics and membrane fluidity. Elevated temperatures can also denature proteins and affect membrane integrity. 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 HT-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 HT-2 toxin (Fig.4). Based on date presented (Table 1) the effect of HT-2 toxin was maximal when yeast cells were cultured at 30 °C. It was observed that HT-2 toxin did not have growth retardation effects below 15 °C. In addition, a heat pulse (55 °C for 5 min) increased the sensitivity of *S. carlsbergensis* to HT-2 toxin by five fold.

CONCLUSIONS

Membrane-modulating agents enhanced the toxic action in wild-type yeast. The membrane plays a significant role in the interaction of HT-2 toxin with yeast cells.

REFERENCES

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