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# PARTIAL CHARACTERISATION OF CHOLERA TOXIN BINDING ON MEMBRANES OF *SACCHAROMYCES BOULARDII*

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## SUMMARY

In the study reported here we demonstrated the presence of specific binding sites for cholera toxin on *S. boulardii* membranes using $^{125}$I-cholera toxin. The $^{125}$I-toxin bound very effectively to the membrane preparation of *S. boulardii* with a minimum detectable concentration of 0.5 nM and saturation being achieved at 4 nM. The binding was displaced by very low concentration of native cholera toxin ($1 \times 10^{-11}$ M). The displacement of $^{125}$I-cholera toxin from the membranes was a rapid phenomenon with 50% of the binding displaced in about 15 min and 50% of the dissociation obtained at a concentration of approximately $0.5 \times 10^{-8}$ M. The present results indicate a possible new prophylactic method against infections, involving oral intake of *S. boulardii* cells to flush cholera toxin or other cholera-like toxins (*Escherichia coli*) from the intestinal ecosystem.

## INTRODUCTION

The non-pathogenic yeast *Saccharomyces boulardii* has been used in many countries for the treatment of antibiotic-induced diarrhoea, *Clostridium difficile*-associated enterocolopathies and to improve the resistance of intestinal ecosystem to bacterial infection (*McFarland* and *Bernasconi*, 1993). One possible protective mechanism is related to the binding ability of bacterial enterotoxins to the yeast membrane, reducing their effects by competition with the natural receptor. Previous results (*Brandão* et al., 1998) obtained in our laboratory demonstrated that the incubation of yeast cells with cholera toxin triggered a cAMP signal that led to an activation of trehalase suggesting specific binding of the toxin to a receptor localised on the yeast membrane. The presence and a partial characterisation of binding sites for cholera toxin on the *S. boulardii* cell surface were demonstrated in the present study, suggesting a possible prophylactic method that would involve oral intake of the yeast to flush cholera toxin or other cholera-like toxins (*Escherichia coli*) from the intestinal ecosystem.

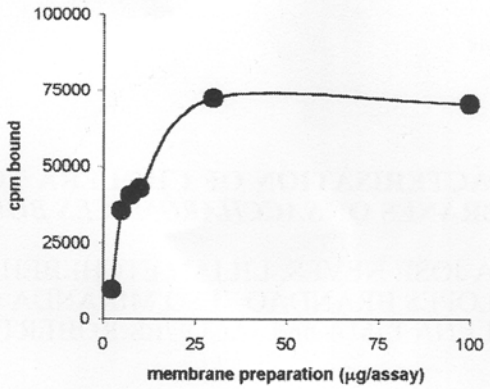


**Figure 1**: Effect of *S. boulardii* membrane concentrations on the binding of $^{125}$I-cholera toxin. Membrane proteins were incubated for 30 min at 37°C with 50 nM of $^{125}$I-cholera toxin.

## MATERIAL AND METHODS

### Radiolabelling of cholera toxin

Cholera toxin was iodinated according to the procedures described by *Brandão* et al. (1998). Radiolabelled toxin is extremely unstable and must be used in no more than 3 days after its preparation.

### Yeast membrane preparations

*S. boulardii* (Floratil®, Merck S.A, Rio de Janeiro, Brazil) was grown at 30°C in 1% of yeast extract, 2% of peptone and 2% of glucose. Stationary phase cells were harvested, and membranes were isolated as described by *Becher* et al. (1992). The concentration of protein is determined by the Lowry method using BSA as standard. Membrane preparations were stored at -20°C for 3 months.

### Experimental procedure

The binding studies with the membrane preparations (5 µg/assay or as mentioned) of *S. boulardii* were carried out in 25 µl buffer (100 mM sodium phosphate buffer pH 7.4 plus 1 mM EDTA, 0.1 mM PMSF and 0.1% BSA) at 37°C for 30 min. The preparations were incubated with radiolabelled toxin (total binding) or with the radiolabelled toxin with excess (100-1000 times) of native toxin (non-specific binding). The total binding minus the non-specific binding gives the specific binding. The free ligand was separated from the bound ligand by rapid vacuum filtration. In the absence of membranes, 5-15 % of the freshly prepared $^{125}$I-toxin was adsorbed onto the cellulose acetate filters. All the data presented here are described in terms of specific binding. The contribution of non-specific binding to the total quantity of radioactive uptake ranged from 30 to 40%.

## RESULTS AND DISCUSSION

The binding of $^{125}$I-toxin (50 nM) was linearly related to the concentration of membrane proteins until 30 µg/assay and there was no evidence for a gradual increase in toxin binding above this concentration (Figure 1). A concentration of 5 µg protein/assay was used in the experiments

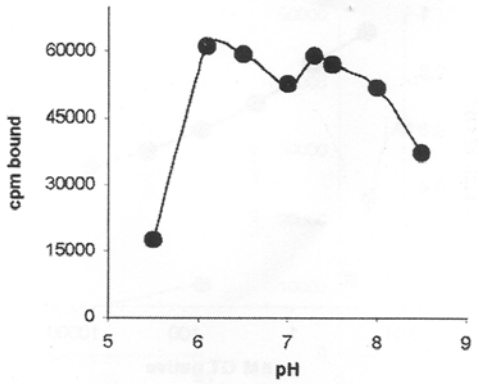


**Figure 2**: Influence of pH on cholera toxin binding to membrane preparations of *S. boulardii*. Incubation (30 min at 37°C with 50 nM of $^{125}$I-cholera toxin) was performed with 30 µg/assay of membrane preparation in buffer. The buffer pH was adjusted to the values indicated. Non specific binding was determined using 44 nM of native cholera toxin.

described. At this concentration, maximum binding observed was always below 10% of the total radioactivity and the availability of $^{125}$I-cholera toxin was never a limiting factor.

Higher toxin binding was observed for pH values ranging from 6.0 to 8.0 with a discrete maximum at pH 6.4. Inhibition of the binding was observed at both acidic and basic pHs, but more accentuated for the former (Figure 2).

As can be seen from Figure 3, $^{125}$I-cholera toxin bound specifically, in a saturatable manner, to the membrane preparation of *S. boulardii*. The saturation binding depended on the concentration of membrane preparation, with 5 µg membrane preparation/assay (Figure 3), the minimum detectable

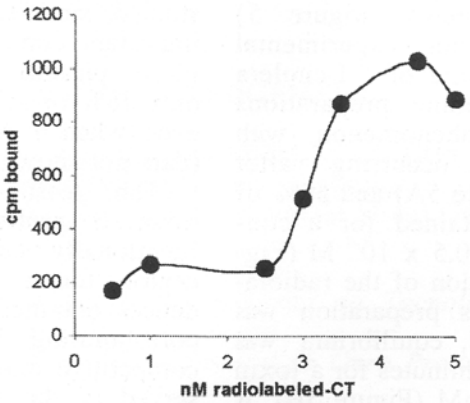


**Figure 3**: Specific binding of cholera toxin to membrane preparation of *S. boulardii*. Membrane preparations (5 µg/assay) were incubated at 37°C for 20 min in the presence of increasing concentrations of $^{125}$I-cholera toxin in the absence (total binding) or in the presence (non-specific binding) of 1,000 times as much native cholera toxin. Specific binding was determined as the difference between total binding minus non-specific binding.

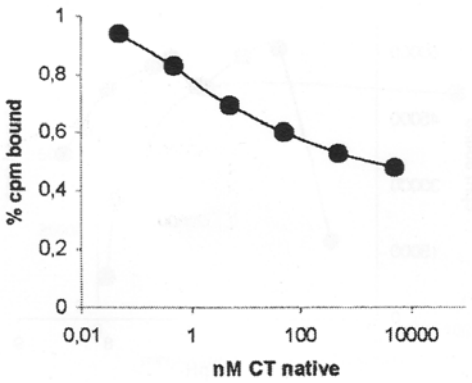


**Figure 4**: Displacement of cholera toxin binding to membrane preparations of *S. boulardii*. Membrane preparations (5 µg/assay) were incubated for 20 min at 37°C with 2 nM $^{125}$Icholera toxin and increasing concentrations of native cholera toxin.

concentration of cholera toxin was 0.5 nM and the saturation achieved at 4 nM.

The binding was displaced by very low concentration of native cholera toxin ($5 \times 10^{-11}$ M) as can be observed from Figure 4. The maximum concentration of native cholera toxin used ($5 \times 10^{-6}$ M) did not displaced totally the $^{125}$I-toxin binding from the membrane preparations. At this concentration, about 50% of $^{125}$I-toxin still remained bound to the membrane preparation (Figure 4).

Kinetics experiments (Figure 5) show that, under our experimental conditions, the binding of $^{125}$I-cholera toxin to the membrane preparations was a reversible phenomenon with 50% dissociation occurring after about 15 min (Figure 5A) and 50% of the dissociation obtained for a concentration of about $0.5 \times 10^{-8}$ M (Figure 4). The association of the radiolabelled toxin to this preparation was rapid and at 37°C, equilibrium was achieved within 10 minutes for a toxin concentration of 4 nM (Figure 5B). A slight decrease in toxin binding was observed when the incubation was prolonged for more than 30 minutes (Figure 5B). Binding at 37°C was higher than at 27°C (data not shown) and this can be an advantage for *S. boulardii* since probiotics, by definition, should perform most effectively at mammalian internal body temperatures.

As an initial characterisation of the structure of toxin binding sites on yeast membranes, competitive binding experiments were performed using galactose. Figure 6 shows that this carbohydrate competed with the $^{125}$I-cholera toxin for the binding sites with an $IC_{50}$ of 0.65 M. Competitive binding using mannose was also studied, since this carbohydrate is an important constituent of the *S. cerevisiae* plasma membrane. However, only 16% of inhibition were observed even when 1 M mannose was used (data not shown).

The yeast receptor for cholera toxin is probably structurally and functionally similar to the GM1 receptor found on enterocyte. Evidences obtained in the present study corroborating this theory include the competitive inhibition of binding observed in the presence of galactose (Figure 6) although there is a considerable difference in $IC_{50}$ values between mammalians cells (45 mM) (*Mertz* et al., 1996) and *S. boulardii* (approximately 650 mM).

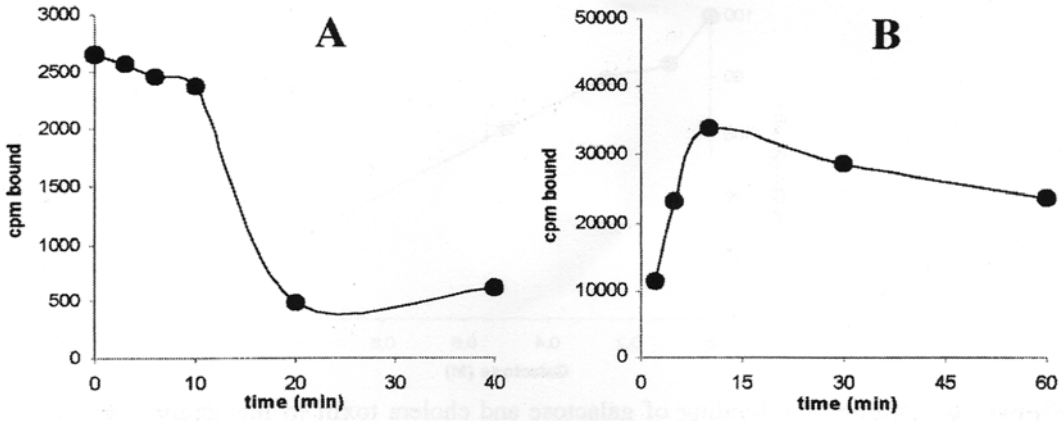


**Figure 5**: Binding characteristics of cholera toxin to membrane preparation of *S. boulardii*.

Part A - Membrane preparations (6 μg/ml) were incubated with 2 nM of $^{125}$I-cholera toxin for 60 min at 37°C.Dissociation was initiated with the addition of 2 μM of native cholera toxin. Samples of 0.5 μg were filtered at the indicated times and specific binding was determined.

Part B - Membrane preparations were incubated at 37°C with 50 nM of $^{125}$Icholera toxin. Samples were collected at the times indicated and the binding of $^{125}$I-cholera toxin to *S. boulardii* membrane preparations determined.

## CONCLUSION

Our results suggest that the activity of cholera toxin (and probably of other toxins) could be neutralised by binding to the *S. boulardii* surface. This binding could reduce the number of toxin molecules available to bind the enterocyte receptors, producing an attenuation of diarrhoea in patients treated with the yeast cells.

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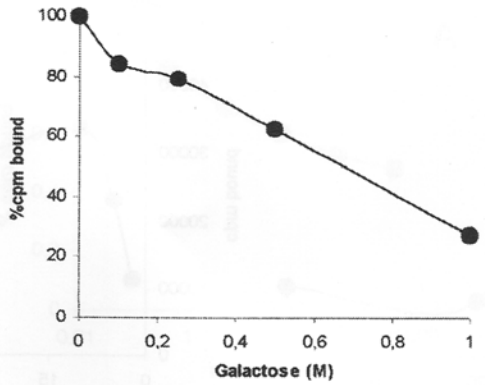


**Figure 6**: Competitive binding of galactose and cholera toxin to membrane preparations *of S. boulardii.*

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