Note

Biofunctional Change in Yeast Cell Surface on Treatment with Triton X-100

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In our studies on fermentative production of sugar nucleotides and cytidine derivatives,^{1~6)} we found that Triton X-100 treatment of yeast cells allowed them to phosphorylate nucleotide monophosphate (CMP) to nucleotide triphosphate (CTP) and then, convert them further to a final nucleotide derivative (CDP-choline). Untreated cells were not able to carry out these reactions. This suggested that some change in the yeast cell surface must occur. Thus, we carried out the following experiments to determine the action of Triton X-100 on yeast cells.

(1) *Optimal conditions for treatment with Triton X-100.* *Saccharomyces carlsbergensis* (IFO 0641) and *S. cerevisiae* (IFO 0635) were cultured in Ballou's medium.^{7,8)} After harvesting at the log phase, the cells were treated with various concentrations of Triton X-100 (see Fig. 1), and the ability to phosphorylate nucleotides was deter-

mined. The methods were as described in previous papers.^{1~6)} Figure 1 shows that when cells were treated with Triton X-100, CMP was phosphorylated to CDP and CTP, and then converted to CDP-choline. Untreated cells could not phosphorylate CMP. The response to the Triton X-100 treatment depended on the yeast species. *S. cerevisiae* required treatment with higher concentrations of Triton X-100. This suggested that *S. cerevisiae* has a stronger or thicker cell surface than *S. carlsbergensis*. Figure 2 shows that after 2 hours of treatment with 0.04% of Triton X-100 the cells of *S. carlsbergensis* can effectively phosphorylate CMP and produce CDP-choline.

(2) *Effect of Triton X-100 on viability of yeast cells.* Since the treatment of cells with Triton X-100 showed a great difference between *S. cerevisiae* and *S. carlsbergensis* in the fermentative production of nucleotide derivatives, we checked the effect of Triton X-100 on the viability of yeast cells. Cells of *S. carlsbergensis* and *S. cerevisiae* were treated with 0.04% Triton X-100 for various times, and then spread on nutrient agar plates followed by incubation at 28°C. As seen in Table I, the treatment did not significantly affect yeast cell viability. These results were further confirmed by growing cells of *S. carlsbergensis* and *S. cerevisiae* in the presence of Triton X-100. No significant killing was observed even with 8% Triton X-100 in the medium (data not shown).

(3) *Observation of cell surface by scanning electron microscope.* The cell surface of yeasts treated with Triton X-100 was observed with a scanning electron microscope. However, as seen in Figs. 3 and 4, no differences were seen between control and treated cells of either species. Although microscopic differences were not detected, the

FIG. 1. Effect of Triton Treatment on Yeasts.

After harvesting, cells were washed 2 times with deionized water, and then treated for 8 hr with various concentrations of Triton X-100 as given on the abscissa axis. After removal of Triton by centrifugation, the treated cells were subjected to analysis for fermentative activity converting CMP to CTP, and then further to CDP-choline. The reaction was carried out for 10 hr.

FIG. 2. Effect of Treatment Time on *Saccharomyces carlsbergensis*.

Cells of *Saccharomyces carlsbergensis* were treated with 0.04% of Triton X-100 for the various times given on the abscissa axis, and then subjected to the analysis as described in the legend of Fig. 1.

TABLE I. EFFECT OF TRITON TREATMENT ON VIABILITY OF CELLS

Treatment time (hr)	Number of viable cells (cells/ml)	
	Control	Triton treatment (0.04%, 8 hr)
(A)	0	4.1×10^8
	4	4.1×10^8
	8	3.8×10^8
	12	3.9×10^8
(b)	0	12.5×10^8
	4	13.2×10^8
	8	12.8×10^8
	12	13.2×10^8

(A) *S. carlsbergensis*; (B) *S. cerevisiae*.

fact that cells treated with Triton X-100 could carry out fermentative production of CDP-choline and other sugar nucleotides suggested that some functional changes in the cell surface may have occurred. Perhaps, the changes are too small to be detected by the scanning electron microscope. Recently, another effect of Triton X-100 was observed. In cooperative work with Dr. Gunge, a plasmid was transformed into yeast cells that had been treated with Triton X-100 (unpublished data). This result is now being confirmed and will be published elsewhere. In sum-

Saccharomyces cerevisiae

Control (without treatment)

Treated with 0.04% Triton X-100 for 8 hr.

FIG. 3. Fine Structure of Cell Surface of *Saccharomyces cerevisiae*.

mary, Triton X-100 treatment may provide an useful method for the enhanced uptake by intact yeast cells of various substances.

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Saccharomyces carlsbergensis

Control (without treatment)

Treated with 0.04% Triton X-100 for 8 hr.

FIG. 4. Fine Structure of Cell Surface of *Saccharomyces carlsbergensis*.

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