ACTION OF ITURIN A ON MEMBRANE VESICLES FROM *SACCHAROMYCES CEREVISIAE*: ACTIVATION OF PHOSPHOLIPASES A AND B ACTIVITIES BY PICOMOLAR AMOUNTS OF ITURIN A

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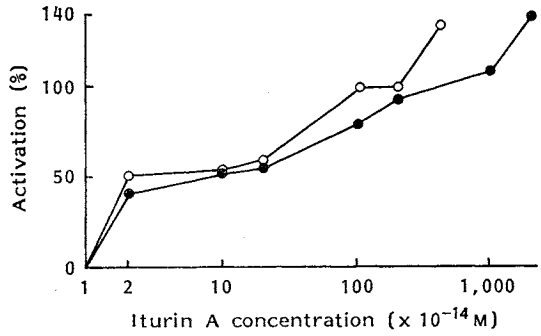
Iturin A is a lipopeptide antibiotic containing a cyclic peptide moiety with a DLDDLLDL sequence and a lateral aliphatic chain[1]. The conformational analysis shows that iturin A seems to have a rather stiff peptide ring surrounded by mobile side chains[2]. This structure makes iturin A able to interact with phospholipids and to cause modifications in the fine structure and physical properties of membranes[3]. The effects of iturin A on *in vivo* phospholipid metabolism in yeast cells have been reported: There is a release of fatty acids from phospholipids of *Saccharomyces cerevisiae*[4]. In this communication, we demonstrate an *in vitro* activation of phospholipid acyl hydrolases (phospholipases) from membrane vesicles of *S. cerevisiae* by iturin A.

S. cerevisiae NCYC 366 was grown at 28°C on a medium containing peptone 1%, yeast extract 0.2% and glucose 2% (w/v). The cells were harvested in the late exponential phase, washed with water and with a solution of 0.1 M glycine - 0.3 M KCl, pH 7.0. This solution was used as osmotic stabilizer throughout the plasma membrane vesicles preparation according to the method of FUHRMAN *et al.* modified by WITT *et al.*[5].

The lipids were labeled by growing yeast with sodium [^{14}C]acetate (1 μCi/ml). The extraction of lipids was carried out by $CHCl_3$ - MeOH (1:1) then by $CHCl_3$ - MeOH (2:1). Crude phospholipids precipitated with acetone had a specific activity of 1.6 μCi/mg. They were fractionated by chromatography on Bio-Sil HA (Bio-Rad). The major fractions, containing a mixture of phosphatidylcholine (96.2%) and lysophosphatidylethanolamine (3.8%) were pooled and used in further assays.

Fig. 1. Activation of phospholipase activity by iturin A.

○ Phosphatidylcholine, ● lysophosphatidylethanolamine.



The plasma membrane vesicles, protein concentration 0.25 mg/ml, were suspended in buffer containing glycine 90 mM, acetic acid 90 mM, NaCl 10 mM at pH 4.0.

Labeled substrate (1 mg) was sonicated for 1 minute at 0°C in 1 ml of acetate buffer (100 mM sodium acetate - acetic acid) pH 4.0; 20 μl of substrate suspension were added to 5 μl of the solution of iturin A at various concentrations. The reaction was initiated by the addition of 30 μl of plasma membrane vesicles suspended in glycine buffer. The final volume of the assays was adjusted to 100 μl with glycine buffer. Controls without antibiotic were carried out simultaneously. After 3 hours incubation at 30°C the lipids were extracted with 0.4 ml $CHCl_3$ - MeOH (2:1) and separated by TLC on silica gel in the solvent $CHCl_3$ - MeOH - water (65:25:4). The spots of phospholipids were scraped and counted for radioactivity. The phospholipase activity was determined by the measure of the disappearance of phospholipids.

The activation of phospholipases was calculated as follows: Phospholipids hydrolyzed after 3 hours incubation in presence of iturin A $= P_1$, in absence of iturin A $= P_2$; activation (%) $= \frac{P_1 - P_2}{P_2} \times 100$.

The results are shown on Fig. 1. An activation of phospholipase activity was observed in the presence of iturin A in a concentration range 2×10^{-14} to 4×10^{-12} M for phosphatidylcholine and 2×10^{-14} to 2×10^{-11} M for lysophosphatidylethanolamine. Higher concentrations of iturin A

gave an opposite effect, the phospholipase activity was inhibited.

The mechanism of activation of phospholipases is yet speculative. The most curious result is the very low concentrations of iturin A which gave this activation. Such low concentrations have been previously found for creating pores in artificial bilayer membranes[3]. There is a large discrepancy between these concentrations and those which are lethal for *S. cerevisiae* cells ($\simeq 20\ \mu M$)[6]. One can suppose that only a small amount of the antibiotic reaches the cell membrane, *in vivo*; this antibiotic was able to induce the formation of pores in the membrane. The activation of phospholipases could be the consequence of the disorganization of the membrane which permits a better interaction between phospholipids and phospholipases.

Acknowledgments

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