PROTON PERMEABILITY INDUCED BY POLYENE ANTIBIOTICS

A plausible mechanism for their inhibition of maltose fermentation in yeast

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1. Introduction

Polyene antibiotics are the most important antifungal agents presently known [1] and their mechanism of action is based on a specific interaction with membrane sterols that results in a changed permeability [2]. The magnitude of these permeability changes is dependent on the concentration of antibiotic and under certain conditions a complete disruption of the yeast permeability barrier can be achieved [3]. The nature of the primary lession of the yeast plasma membrane caused by low concentrations of polyene antibiotics has not been established although loss of potassium from the cells seems to be one of the primary effects of these antibiotics [3,4].

We have found that maltose fermentation in *Saccharomyces cerevisiae* is inhibited by the polyene antibiotics nystatin and amphotericin B. Our studies on the mechanism of this inhibition prompt us to propose as a working hypothesis that the primary event produced by the polyene antibiotics is to increase the proton permeability of the yeast plasma membrane.

2. Methods

Saccharomyces cerevisiae (strain S-13 gal) was grown in a maltose-rich medium and harvested as in [5].

The cells were treated with the appropriate amounts of polyene antibiotics at 100 mg (wet wt)/ml and the medium was buffered with 0.1 M 2-(*N*-morpholino) ethane sulfonic acid, adjusted to pH 6.4 with Tris. Incubation was for 80 min at room temperature to obtain a maximum effect. Aliquots of these suspensions were used for the determination of different metabolic parameters.

Stock solutions of nystatin (Squibb, 3800 units/mg) and amphotericin B (Sigma) were prepared in dimethyl sulfoxide and the control incubations without polyenes received the same amount of this solvent (less than 2%) to correct for possible effects on the metabolic parameters studied.

Sugar fermentation was measured by conventional manometric techniques in a medium containing 0.1 M 2-(*N*-morpholino) ethane sulfonic acid, adjusted to pH 6.4 with Tris, 50 mM glucose or maltose and 10 μg/ml antimycin A to inhibit respiration. Yeast concentration was 10 mg/ml and when indicated 0.1 M KCl was also included.

For the measurement of sugar transport into the cells, 50 μl aliquots treated cells were mixed with 5 μl either [U-^{14}C]maltose (40 mM and 0.1 Ci/mol) or [U-^{14}C]xylose (200 mM and 0.2 Ci/mol). After 20 s incubation at room temperature transport was stopped with cold water and the radioactivity in the filtered cells determined as in [5]. Xylose is a non-metabolizable sustrate of the glucose transport system [6]. The hydrolysis of *p*-nitrophenyl-α-glucoside was measured as in [7].

For the experiments where the proton permeability was measured the cells were centrifuged free of buffer and resuspended at 20 mg/ml in a medium containing 125 mM KCl and 50 μg/ml antimycin A. After 2 min 50 mM 2-deoxyglucose were added to trap residual ATP. The suspension was placed in a water-jacketed vessel and the pH changes were recorded as in [5]. The temperature of the circulating water was 14°C.

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3. Results and discussion

Nystatin and amphotericin B inhibit glucose and maltose fermentation in yeast (table 1). However, two important differences are observed between the inhibition of these two fermentations. Maltose fermentation is much more sensitive to low doses of nystatin than glucose fermentation and is not affected by the presence of KCl, while the inhibition of the glucose fermentation is greatly reduced in the presence of 0.1 M KCl.

The results obtained with glucose are in accordance with [3] and indicate that the inhibition of glucose fermentation is caused basically by the loss of potassium from the cells. Maltose fermentation seems to be inhibited by a different mechanism which is operative at lower doses of nystatin than those required for the potassium mediated effects.

As maltose hydrolysis is not affected by the polyene antibiotics (see values in table 2), the only metabolic step which could be responsible for the differences in the inhibition of glucose and maltose fermentation is the transport across the yeast plasma membrane. Glucose transport proceeds by a facilitated diffusion while maltose transport is an active process coupled to the proton gradient [5]. Therefore we investigated the effect of polyenes on both transport systems. The results in fig.1 indicate that maltose transport is more inhibited by amphotericin B than the transport of the non metabolizable glucose analog xylose. The fact

Table 1
Differential inhibition of glucose and maltose fermentation by polyene antibiotics

Sugar	Polyene antibiotics (μg/mg yeast)	Fermentation rate (nmol CO_2/min × mg yeast)	
		Without added KCl	With 0.1 M KCl
Glucose	None	53	53
	Nystatin (0.05)	44	48
	Nystatin (0.13)	32	48
	Nystatin (0.54)	3	38
	Amphotericin B (0.54)	3	30
Maltose	None	44	43
	Nystatin (0.05)	9	9
	Nystatin (0.13)	8	6
	Nystatin (0.54)	< 2	< 2
	Amphotericin B (0.54)	< 2	< 2

Table 2
Hydrolysis of *p*-nitrophenyl-α-glucoside by yeast cells treated with polyenes

Polyenes (μg/mg) yeast	Hydrolysis of *p*-nitrophenyl-α-glucoside (nmol/min × mg yeast)
None	< 3
Amphotericin B (0.65)	< 3
Nystatin (0.65)	< 3
Nystatin (40)	7
Nystatin (100)	69
Yeast cells permeabilized with toluene [8]	70

Yeast cells were treated with the indicated doses of polyenes and the hydrolysis of *p*-nitrophenyl-α-glucoside was measured as in [7]

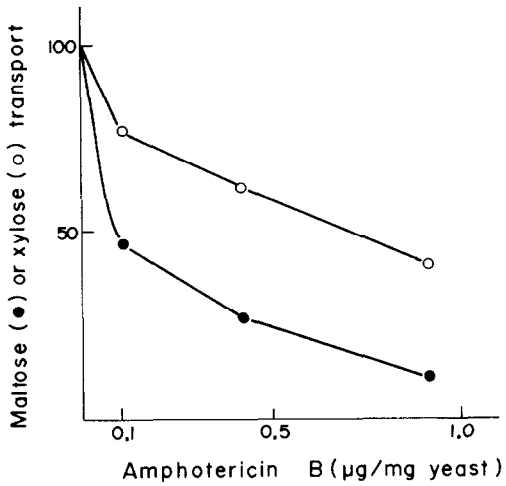

Fig.1. Inhibition of maltose and D-xylose transport by amphotericin B. Yeast cells were treated with the indicated amount of amphotericin B and the transport of maltose (•) and D-xylose (○) was measured as in section 2. The results are expressed as % control without amphotericin B. These controls amounted to 2.8 nmol maltose/min × mg yeast and 7.4 nmol D-xylose/min × mg yeast.

that the transport of a pentose is less inhibited than the transport of the much bigger maltose molecule suggests that most of the inhibition is not caused by disruption of the membrane and leakage of the transported sugar during the washing of the cells.

Further evidence against non specific permeability changes caused by the polyenes in our experimental conditions was obtained by measuring the hydrolysis of *p*-nitrophenyl-α-glucoside in whole cells. This glucoside is good sustrate for the intracellular maltase but is not transported by the maltose transport system. Accordingly, yeast cells would not hydrolyze *p*-nitrophenyl-α-glucoside unless their permeability barrier has been disrupted. As shown in table 2 the polyenes only make yeast cells permeable to the glucoside when concentrations 2 orders of magnitude higher than those required to inhibit transport are employed.

It has been shown that maltose transport is inhibited by uncouplers and coupled to the uptake of protons by the cells [5]. This indicates that its driving force is the electrochemical gradient of protons. A plausible mechanism for the inhibition of maltose fermentation caused by the polyenes would be that these antibiotics increase the permeability of the membrane for protons and dissipate the proton gradient necessary for maltose transport.

In order to test this hypothesis we have studied the effect of low doses of amphotericin B on the proton permeability of yeast cells.

The cells were depleted of ATP by treatment with antimycin A and 2-deoxyglucose [5] in order to suppress proton movements supported by metabolism. When the recorded pH stabilized, a pulse of acid was added and the time course of the pH changes was measured (fig.2). The initial pH drop caused by the acid is partially reversed as the protons penetrate inside the cells and the time course of this reversion is a measure of the proton permeability of the membrane [9]. Control cells have a very low proton per-

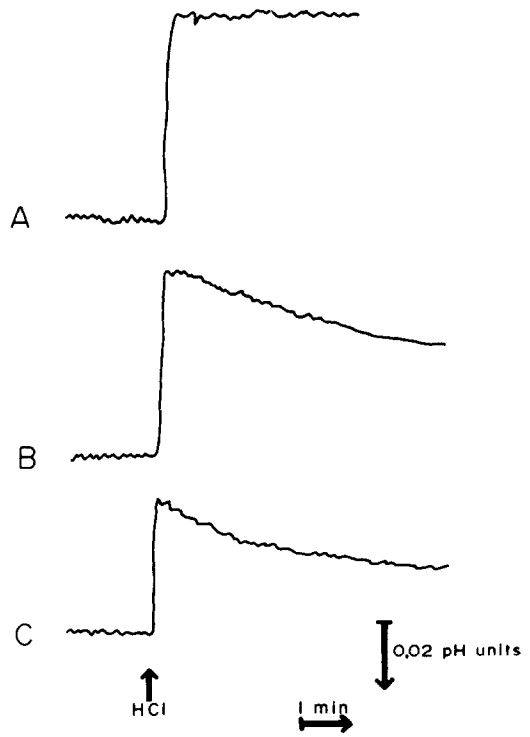

Fig.2. Effect of dinitrophenol and amphotericin B on the proton permeability of ATP depleted yeast cells. Yeast cells were treated as in section 2 in the absence (A and B) or in the presence of 0.5 μg amphotericin B/mg cells (C). They were depleted of ATP by the addition of antimycin A and 2-deoxyglucose. In exp. B 0.3 mM dinitrophenol was also included. A pulse of 50 nmol HCl was added as indicated. The starting pH values were 6.50 (A), 6.44 (B) and 6.28 (C).

meability (fig.2A) which is greatly increased by the proton conductor dinitrophenol (fig.2B). This indicates that the movement of protons is not limited by the movement of some other ion as for example potassium. The treatment of the cells with amphotericin B results in increased proton permeability in accordance with the above-suggested mechanism (fig.2C).

The chemiosmotic theory of Mitchell has been very fruitful for the understanding of energy transduction in mitochondria, chloroplasts and bacteria [10,11]. Recently, the active transport processes of eukaryotic microorganisms have been interpreted according with this theory [5,12–15].

Taking into account the central role of the proton gradient in cellular membranes, the dissipation of this gradient could be the basis for an antibiotic effect at the membrane level. When the cellular membranes are altered by such antibiotics as polyenes, protons could be the species whose permeability would be firstly increased, due to its smaller size and greater mobility in solution. The increase in proton permeability would collapse the proton gradient and therefore the uptake of nutrients coupled to this gradient would be blocked. This effect would be observed at concentrations of the polyenes much lower than required to disrupt the permeability barrier for larger molecules.

With these data it is possible to explain not only the inhibition of maltose transport but also the loss of potassium caused by these antibiotics. Although the most obvious mechanism for this exit of potassium is the formation of ionic channels in the membrane [16,17], the fact that it does nor occur significantly at 0°C [18,19] argues against this explanation. Alternatively, it has been shown that potassium accumulation in yeast is coupled to the proton gradient and when this gradient is dissipated by uncouplers potassium is lost from the cells [20]. Therefore, the loss of potassium caused by the polyenes could be a consequence of the uncoupling effect of these antibiotics at the level of the plasma membrane.

In conclusion it can be said that the hypothesis that the primary effect of the polyene antibiotics is the dissipation of the proton gradient should be considered in future studies on these and other antibiotics acting on the membranes such as the polymyxines [21].

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