Influence of Spermine on Some Membrane-disturbing Actions

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Spermine, Yeast Cells, Membrane Damage

The influence of spermine on the potassium loss from yeast cells as a consequence of membrane-disturbing actions was investigated. Spermine interfered strongly in the action of membrane-active bactericides on yeast cells; this interference resembles the action of certain metal ions. Spermine provides a protection against the positively charged pararosaniline and accomplishes a strong potentiation of the action of the negatively charged sodium dodecyl sulphate.

Polyamines like spermine are widespread occurring compounds in several living organisms [1, 2]. They seem to be necessary for the growth of a number of micro-organisms [1], but on the other hand they possess an antibacterial activity, though for the latter action metabolic activity of the cell is necessary [3, 4]. An interesting property of these amines is their ability to prevent lysis of osmotic sensitive forms – protoplasts or spheroplasts – of a number of bacteria, in which process the cytoplasmic membrane of the cell is assumed to play an important role [5–7]. In these investigations the decrease in optical density, *i. e.* complete desintegration of the whole cell, was considered as a measure for the degree of destabilization.

In our investigation we have considered the protective action of some polyamines, especially spermine, with regard to a more subtle aspect of membrane damage, measuring potassium leakage from yeast cells as a measure for loss of membrane impermeability. The yeast cells, or protoplasts therefrom, were exposed to a number of actions, which are known to induce potassium leakage and the action of spermine thereupon was examined.

Materials and Methods

As a testorganism yeast, strain *Saccharomyces cerevisiae* NCYC 983, was used. A stocksuspension was prepared containing 10^9 cells per ml. Protoplasts were prepared from log phase cells with snail gut juice, in 0.8 M mannitol as stabilizing medium, as previously described [8]. Here too a stocksuspension with 10^9 cells per ml was prepared.

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Addition of 4.5 ml water to 0.5 ml protoplast-suspension resulted in complete loss of intracellular potassium.

To 4.5 ml reaction mixture 0.5 ml stocksuspension was added. Protoplasts were used in the experiments in which potassium leakage was considered as a function of the tonicity of the medium; in the other experiments whole yeast cells were used. After ten min the suspension was centrifuged and in the supernatant potassium was estimated by flame photometry.

Results and Discussion

A first experiment was done with yeast protoplasts because it was known from literature that polyamines could prevent lysis of osmotic sensitive forms of certain bacteria. When protoplasts are placed in a medium of increasing hypotonicity leakage of potassium occurs. Spermine (10^{-3} M) did not afford any protection with regard to this kind of membrane damage: The same potassium loss was observed in the presence or absence of spermine.

A comparative result was obtained in an experiment in which the membrane permeability was measured as a function of temperature. Suspensions of yeast cells, incubated at elevated temperatures, lose potassium, which loss is increased when the temperature is elevated. Here too no protection was produced by spermine; potassium leakage is not different with or without spermine. Cations like Ca^{2+} did also have no influence.

A very clear effect of polyamines was observed with regard to the action of positive and negative bactericides. Spermine strongly inhibits the action of the dye pararosaniline on yeast (Fig. 1). This

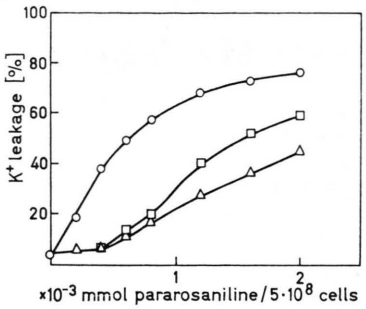

Fig. 1. Leakage of potassium ions from yeast cells as a consequence of the action of the dye pararosaniline and the influence of spermine there upon. —○—, without spermine; —□—, with $4 \cdot 10^{-5}$ M spermine; —△—, with $4 \cdot 10^{-4}$ M spermine. Potassium leakage was expressed as percentage from maximal leakage, as determined by boiling an aliquot of yeast cells.

positively charged dye induces membrane damage and potassium leakage at low concentrations [9, 10]. Spermidine was somewhat less effective than spermine whereas putresceine required a much higher concentration to afford protection. This protective action has also been observed with metal ions; the degree of effectiveness depends on the nature of the metal ion [11, 12]. Spermine proved to be more effective than Ca^{2+} but less effective than heavy metal ions like La^{3+}.

There is also a strong action of spermine with regard to sodium dodecyl sulphate (SDS) but here the action is a reverse one as compared with pararosaniline. Negatively charged bactericides like SDS generally have a poor action on yeast cells [13] and bacteria. Spermine does greatly enhance the effect of SDS, as can be seen in Fig. 2. Here the effect of spermine is very pronounced; it is even more effective than the heavy metal ion La^{3+}. Spermidine is less active than spermine but also more active than La^{3+}, whereas putresceine and Ca^{2+} show a poor potentiating effect.

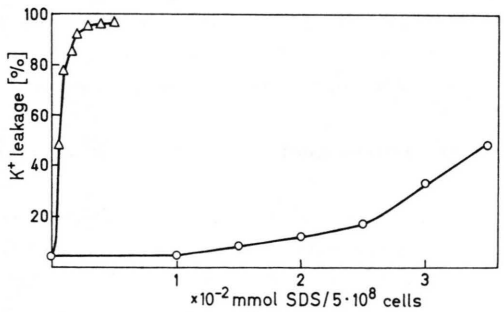

Fig. 2. Potentiation of the membrane-damage inducing effect of sodium dodecyl sulphate (SDS) by spermine. —○—, potassium leakage without spermine present and —△—, in the presence of $4 \cdot 10^{-4}$ M spermine. In this case no perceptible potentiating effect was observed in the presence of $4 \cdot 10^{-5}$ M spermine.

A reasonable explanation of these effects is based on the observed strong interaction of spermine with phosphate groups; the compound is bound to the yeast cell [1, 3, 4]. In the binding of the positively charged dye these phosphate groups — negatively charged phospholipids and polyphosphates in or near the membrane [13, 14] — play an important role. Like metal ions as Ca^{2+} and La^{3+} spermine does not directly induce a change in permeability of the membrane, even not in high concentration, which underlines our formerly formulated hypothesis [12], that mere binding of a compound does not induce membrane damage, but that a hydrophobic part of sufficient length is necessary to penetrate the glycerol moieties of the lipid bilayer. Polyamines like solapalmitine, which is structurally related to spermine but possesses one longer hydrophobic tail, indeed have been shown to disturb membrane impermeability [15, 16]. The binding of spermine or metal ions to phosphate groups competes with the binding of the dye pararosaniline and affords in this way a protection of the yeast cell against dye action.

In the case of the negatively charged bactericides as SDS the binding must be ascribed to the interaction of the hydrophobic tail of the molecule with the hydrophobic interior of the membrane. The negative charge of phospholipids and polyphosphates inhibits strongly the binding of the negatively charged SDS [13]. Spermine, and the metal ions too, are able to mask the negative phosphate groups, thereby enabling the accessibility for SDS to the membrane.

These experiments show that with regard to a number of membrane disturbing actions polyamines behave like metal ions: they regulate these actions by interfering with the negative charges on the membrane.

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