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LOCALIZATION OF POLYPHOSPHATES AT THE OUTSIDE OF THE YEAST CELL PLASMA MEMBRANE

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Under appropriate experimental conditions toluidine blue is bound to the yeast cell surface, without penetrating into the cells. Based on experimental observations it is highly probable that the dye is bound to polyphosphates, localized outside the plasma membrane. The probable localization of polyphosphates outside the plasma membrane is important in the context of the proposed involvement of polyphosphates in glucose transport in yeast.

Introduction

The physiological role of polyphosphates in various microorganisms is still a matter of dispute. A role in the regulation of phosphorus and energy metabolism is well established [1] and a possible role in the accumulation of certain basic compounds, like arginine, has been suggested [2]. It has also been postulated that polyphosphates may play an essential role in active glucose transport in yeast [3]. This hypothesis was based on several lines of circumstantial evidence, indicating that a polyphosphate fraction, localized outside the yeast plasma membrane, is directly involved in transport-associated glucose phosphorylation. In accordance, pulse-labeling experiments provided convincing evidence for glucose phosphorylation during transport in yeast cells [4,5].

Further, the possible localization of polyphosphates outside the plasma membrane is supported by several experimental observations [1,6–11], but unequivocal proof is still lacking. Therefore in recent studies the proposed peripheral localization of a polyphosphate fraction in yeast was reinvestigated. In this context the interaction of the cationic metachromatic dye toluidine blue with yeast cells was studied. The results are presented in this communication.

Materials and Methods

Saccharomyces fragilis was grown, with glucose as carbon source, as described before [12]. The yeast was harvested and washed after 20 h, just before reaching the stationary phase. Phosphate-deficient yeast was obtained by omitting phosphate from the liquid culture medium.

Yeast suspensions were made in distilled water at a final concentration of 0.5% (wet weight). Separation of cells and medium was done by fast centrifugation, utilizing an Eppendorf centrifuge type 3200. Leakage of K^+ into the medium was measured by flame photometry.

Absorption spectra were recorded with an Aminco DW 2 spectrophotometer and corrected for absorption by the yeast cells. Recordings were completed within 15 s. All measurements on yeast were performed after 1 min incubation time. Liposomes of yeast phospholipids were prepared according to Demel et al. [13]. Toluidine Blue 0 ('reinst') was obtained from Serva and was 85% pure on nitrogen basis. Concentrations were corrected for impurity. RNA (from torula yeast, grade IV), DNA (from calf thymus, type I) and polyphosphate (mean chain length = 15) were obtained from Sigma, whereas

yeast mannan was purchased from K and K Laboratories.

Results

Toluidine blue in aqueous solution exhibits a concentration-dependent absorption spectrum, due to monomer (λ_{max} 632 nm) - dimer (λ_{max} 590 nm) equilibrium. Fig. 1 shows the absorption spectrum of a 15 μM dye solution. The absorption maximum at about 610 nm at this toluidine blue concentration is in accordance with spectral data of Michaelis [14]. After addition of yeast cells to the solution a colour shift from blue to purple was observed immediately. As shown in Fig. 1A the absorption was shifted to 545 nm. Such a metachromatic shift of the absorption maximum to lower wavelengths is characteristic for binding of the dye to a negatively charged polyelectrolyte [15,16]. With phosphate-deficient yeast a similar, but much less pronounced metachromatic shift was observed (Fig. 1A).

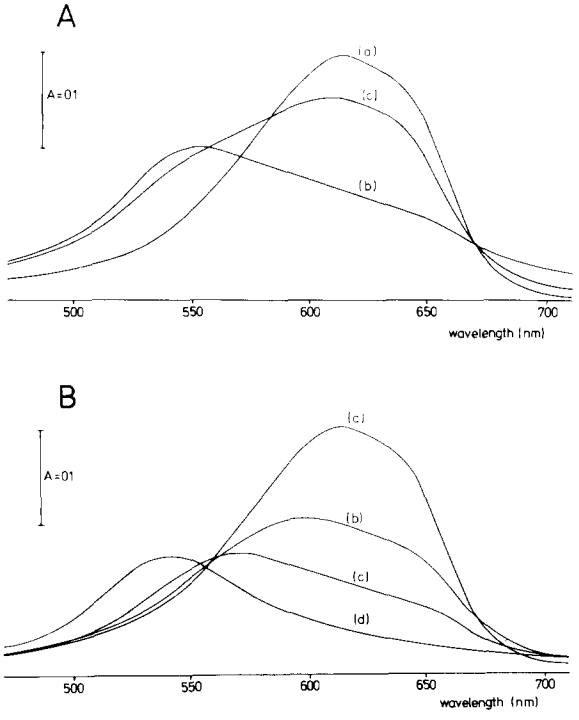

Fig. 1. (A) Absorption spectrum of: (a) 15 μM toluidine blue, (b) 15 μM toluidine blue + 0.5% yeast, (c) 15 μM toluidine blue + 0.5% phosphate deficient yeast. All spectra were recorded after 1 min incubation at pH 4.7. (B) Absorption spectrum of: (a) 15 μM toluidine blue, (b) 15 μM toluidine blue + 16.5 μg/ml RNA, (c) 15 μM toluidine blue + 3.5 μg/ml DNA, (d) 15 μM toluidine blue + 25 μg/ml polyphosphate. All spectra were recorded at pH 4.7.

Non-penetrating cations, like UO_2^{2+}, instantaneously superseded the toluidine blue binding to the yeast cells, as reflected by the disappearance of metachromasia and the re-appearance of the dye molecules in the medium. The effectiveness of various metal ions in this respect was quite different however, as shown in Fig. 2. The observed sequence is characteristic for interaction with a phosphate-containing polyelectrolyte. Polyelectrolytes containing either sulphate or carboxylic groups exhibit quite different sequences [17].

In attempts to determine the chemical nature of the phosphate groups at the yeast cell surface it was found that phospholipids did not cause a metachromatic shift of the toluidine blue absorption spectrum, as observed by addition of liposomes to the dye solution. Also titration of the toluidine blue solution with yeast mannan containing 0.2% phosphorus, did not result in a metachromatic shift, even when the titration was continued to a final phosphate concentration exceeding the dye concentration. Nucleic acids did induce metachromasia, but with a considerably smaller shift of the absorption spectrum. At the optimal nucleic acid/dye ratio a shift to about 570 nm with DNA and to about 590 nm with RNA was found (Fig. 1B), in agreement with the data of Mello [16]. Inorganic polyphosphate, on the other hand, induced a maximal shift to 545 nm (Fig. 1B), as found with yeast cells.

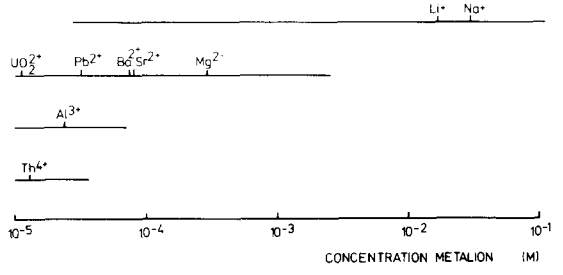

Fig. 2. Concentration metalion giving 50% reduction in toluidine blue binding. (17.5 μM toluidine blue, 0.5% yeast. pH about 4.7).

In experiments, designed to quantitate the number of toluidine blue binding sites at the outside of the yeast cells, it appeared that higher dye concentrations damaged the plasma membrane. This was shown both by K^+ leakage from the cells (Fig. 3A) and by a sudden increase of toluidine blue binding, with a concomitant decrease of the dye concentration in the medium (Fig. 3B). Above a threshold cytolytic concentration toluidine blue apparently penetrates into the cells with subsequent binding to intracellular constituents. Both K^+ leakage and toluidine blue penetration into the cells could be prevented by addition of 20 μg/ml RNA to the medium, up to a dye concentration of 42 μM. Under these experimental conditions toluidine blue binding to the cell surface leveled off at 2.3 μmol/g yeast (Figs. 3A and B). Similar experiments with phosphate-deficient yeast revealed a maximal binding to the cell surface of 1.4 μmol/g yeast.

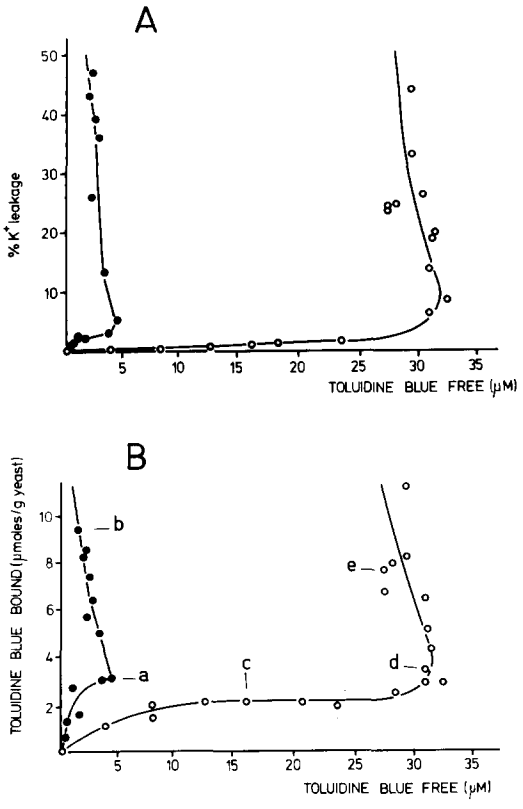

Fig. 3. (A) K^+ leakage caused by toluidine blue. On the abscissa the final free toluidine blue concentration in the medium is plotted. •———•, 0.5% yeast, pH 4.7; o———o, 0.5% yeast + 20 μg/ml RNA, pH 4.7. (B) Bound toluidine blue against the free toluidine blue concentration in the medium. For experimental conditions and symbols see legend to (A). The total initial dye concentration was: (a) 20 μM, (b) 50 μM, (c) 25 μM, (d) 50 μM, (e) 65 μM.

Discussion

At low dye concentrations toluidine blue is bound to negatively charged groups at the outside of the yeast cell membrane, without penetrating into the cells. This can be deduced from the following observations. (1) The binding reaches a maximal value within a few seconds. Moreover, the metachromatic shift of the absorption spectrum, characteristic for binding to a negatively charged polyelectrolyte, is observed instantaneously after adding yeast cells to the dye solution. (2) Non-penetrating cations (like UO_2^{2+}, see Ref. 3) immediately supersede the dye from the binding sites.

Higher toluidine blue concentrations are cytolytic, as indicated by K^+-leakage and strongly increased toluidine blue binding, apparently to intracellular binding sites. Cytolysis can be prevented by addition of RNA to the medium, presumably by complexing unbound dye molecules. Under these conditions maximal binding reaches a level of 2.3 μmol/g yeast. The cytolysis should be distinguished from photodynamic lysis by toluidine blue, as described by Ito and Kobayashi [18] and Ito [19]. These photodynamic effects develop much slower at moderate light intensity. Moreover, when the described experiments were performed in the dark, the results were identical.

The described results strongly suggest that the binding sites at the cell surface are polyphosphates. The much lower binding to phosphate-deficient yeast and the efficiency sequence of ions in superseding toluidine blue as depicted in Fig. 2 indicate a major role of phosphate groups in dye binding. Of the naturally occurring phosphate-containing polyelectrolytes polyphosphate caused a metachromatic shift of the absorption maximum to 545 nm, whereas nucleic acids caused shifts to 570–590 nm (Fig. 3). These different effects can be attributed to the much higher charge density of polyphosphates as compared to nucleic acids. The dependency of the magnitude of the metachromatic shift on charge density has been

discussed e.g. by Sylvén [15]. In connection with these model experiments the metachromatic shift to 545 nm after addition of yeast cells to the toluidine blue solution (Fig. 1A) strongly indicates binding of the dye to polyphosphate binding sites.

It seems very likely that this polyphosphate fraction outside the plasma membrane provides a major contribution to the surface charge of yeast cells. In this connection it is noteworthy that in a recent paper Theuvenet and Borst-Pauwels concluded that probably mainly phosphoryl groups determine the surface charge of yeast cells [20].

Previous observations from our laboratory suggested an essential role of a polyphosphate fraction at the cell surface in glucose transport in yeast [3]. Umnov et al. [21] described experimental evidence indicating a similar role of polyphosphates in glucose transport in *Neurospora crassa*. In this context the results described in this communication are important, as they support the supposed localization of a polyphosphate fraction outside the plasma membrane of yeast cells.

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