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DETECTION OF A YEAST POLYPHOSPHATE FRACTION LOCALIZED OUTSIDE THE PLASMA MEMBRANE BY THE METHOD OF PHOSPHORUS-31 NUCLEAR MAGNETIC RESONANCE

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SUMMARY: Non-penetrating cations, like UO_2^{2+} and Eu^{3+}, are bound to the outside of yeast cells in a reversible fashion. Binding of these ions was attended with a decrease of the ^{31}P NMR polyphosphate signal. Subsequent addition of EDTA to the suspension restored the original spectrum. These experiments confirm the localization of a polyphosphate fraction outside the plasma membrane of yeast.

For the uptake of glucose and glucose derivatives in yeast a transport-associated phosphorylation mechanism has been proposed (1-3). Circumstantial experimental evidence suggested that both in *Saccharomyces* (4) and in *Neurospora crassa* (5) a polyphosphate fraction, localized outside the plasma membrane, is directly involved in this mechanism. This peripheral localization of a polyphosphate fraction in yeast, as predicted by this hypothesis, is supported by several experimental obseryations (6-10).

In model studies on polyphosphate granules, isolated from *Micrococcus lysodeikticus*, it has been shown that the ^{31}P nuclear magnetic resonance (^{31}P NMR) signal of polyphosphates is strongly affected by divalent cations (11). These observations suggested that a study of the influence of non-penetrating cations on the ^{31}P NMR signal of yeast might be used as a non-invasive method for probing the polyphosphate localization in this organism. As described in this communication these studies provided direct evidence for the presence of polyphosphate outside the plasma membrane of *Saccharomyces fragilis*.

METHODS

Saccharomyces fragilis was grown on glucose as carbon source as described before (12). All experiments were carried out at 4°C. In cation binding studies a 5% (wet weight/volume) yeast suspension in distilled water, adjusted to pH 4.0 with HCl, was incubated with varying concentrations of UO_2^{2+} or Eu^{3+} nitrate. The pH remained constant during the experiments. Binding of UO_2^{2+} and

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Eu^{3+} was calculated from the disappearance of these ions from the medium. UO_2^{2+} was measured according to Francois (13), Eu^{3+} according to Rinehart (14). K^+ was assayed by flame photometry.

Damaged cells were stained with uranyl nitrate-Ponceau red (15) or with bromophenol blue (16). Disruption of cells was accomplished by heating to 100°C during 5 min.

For ^{31}P NMR measurements 80% (w/v) yeast suspension, containing 20% D_2O were prepared at pH 4.0. This pH appeared to remain constant during the measurements. Spectra were obtained in the Fourier-transform mode at 81.01 MHz on a Bruker WP 200 spectrometer with a wide bore probe head. The chemical shifts were measured with tetraphenyl phosphonium as an external standard. A relaxation delay of 1 sec was used. In control experiments it appeared that variation of the relaxation delay between 0.1 and 10 sec had no influence on the yeast ^{31}P NMR spectrum.

RESULTS AND DISCUSSION

Binding of UO_2^{2+} and Eu^{3+} to yeast cells is shown in Fig. 1. This interaction exhibits the following characteristics. Binding is independent of the incubation time (2-30 min) and saturable, leveling off at about 2.4 μequiv/g yeast. With disrupted cells the binding was two orders of magnitude higher. Ponceau red and bromophenol blue staining indicated that no membrane damage was provoked by UO_2^{2+} and Eu^{3+} in the concentration range, used in these experiments. The number of stainable cells was less than 1%, under all experimental conditions. Also, no K^+ leakage to the medium was observed. Finally 2.5 mM EDTA reversed UO_2^{2+} and Eu^{3+} binding instantaneously, as reflected by the reappearance of the ions in the medium. In control experiments it appeared that EDTA, as used under the present experimental conditions, did not cause membrane damage, as judged from Ponceau red staining and measurements of K^+-leakage. These characteristics indicate that binding of UO_2^{2+} and Eu^{3+} occurred outside the plasma membrane, without inflicting damage to the diffusion barrier.

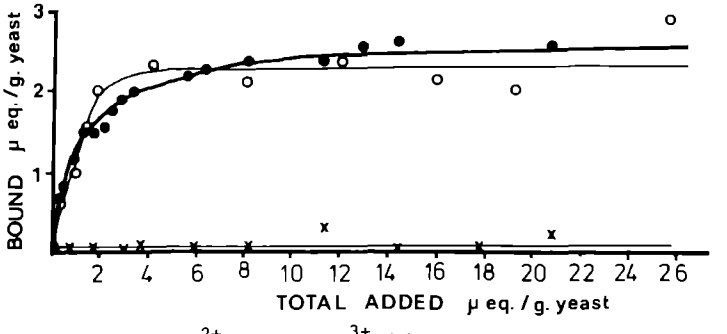

Fig. 1. Binding of UO_2^{2+} (●) and Eu^{3+} (○) to intact yeast cells at 0°C. X: Binding of UO_2^{2+} in the presence of 2.5 mM EDTA.

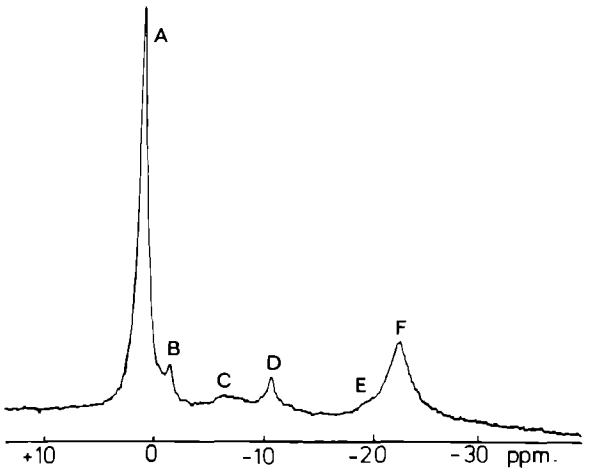

Fig. 2. ^{31}P NMR spectrum of *Saccharomyces fragilis*, in the absence of metabolizable substrate. Peak assignment: A: orthophosphate; B: phosphodiester; C: end-phosphate groups of ATP, ADP and other nucleosides; D: α-phosphate groups of nucleoside phosphates; E: β-phosphate group of ATP and other nucleoside triphosphates and penultimate groups of polyphosphate; F: middle phosphate groups of polyphosphate.

Ponceau red staining and K^+ determinations subsequent to ^{31}P NMR measurements on intact yeast cells demonstrated that also under these experimental conditions the cells were not damaged. A typical ^{31}P NMR spectrum of *Saccharomyces fragilis* is shown in Fig. 2. Peak assignments were made, based on model experiments and literature data (11,17,18). With disrupted cells a similar pattern was obtained.

Titrations of disrupted yeast cells with UO_2^{2+} resulted in a progressive decrease of all spectral lines. With 15 μequiv UO_2^{2+}/g yeast peaks C and F had disappeared completely (Fig. 3). With intact cells quite different results were obtained. After addition of 10-15 μequiv UO_2^{2+} peak F (the polyphosphate signal) had decreased to about 80% of the initial value, without any change of the other signals. With higher concentrations of this ion the polyphosphate signal did not change any further, but peak B decreased, completely disappearing with 30 μequiv UO_2^{2+}/g yeast (Figs 3 and 4). In similar experiments, utilizing Eu^{3+} instead of UO_2^{2+}, identical results were obtained. With reference to the inability of UO_2^{2+} and Eu^{3+} to penetrate into the cytoplasm under these experimental conditions, these observations mean that peak B and part of peak F

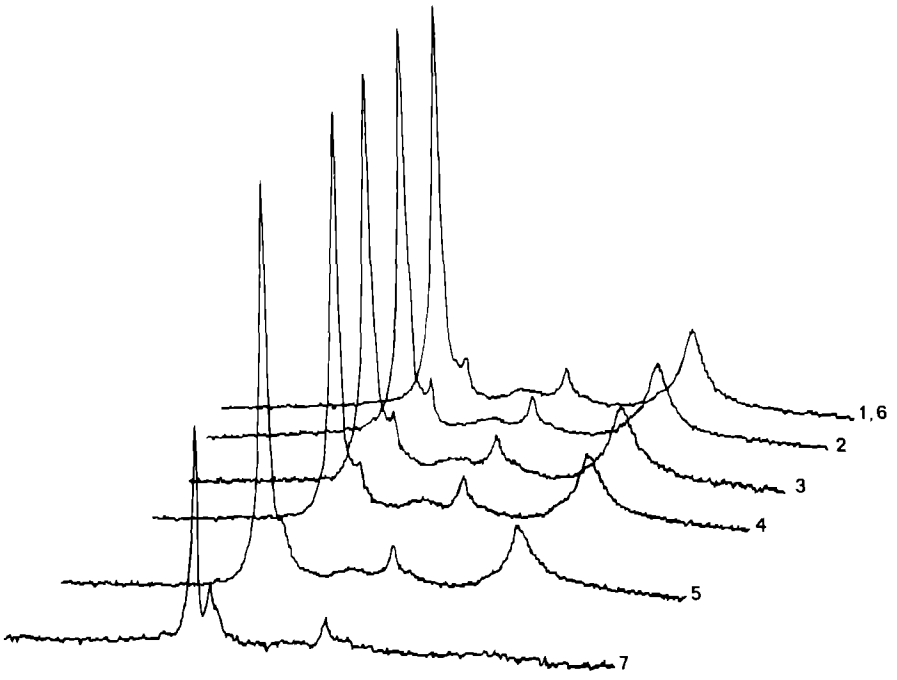

Fig. 3. ^{31}P NMR of *Saccharomyces fragilis*, under different experimental conditions. 1: no additions; 2: 5 µequiv UO_2^{2+}/g yeast added; 3: 10 µequiv UO_2^{2+}/g yeast added; 4: 15 µequiv UO_2^{2+}/g yeast added; 5: 30 µequiv UO_2^{2+}/g yeast added; 6: as 5, followed by addition of a 5-fold molar excess of EDTA; 7: disrupted cells, after addition of 15 µequiv UO_2^{2+}. Titration of intact cells with Eu^{3+} yielded similar results.

originate from outside the plasma membrane. This was confirmed by the fact that EDTA (in a five-fold molar excess with respect to UO_2^{2+}) immediately restored the original spectrum, recorded prior to addition of UO_2^{2+}(Figs 3 and 4).

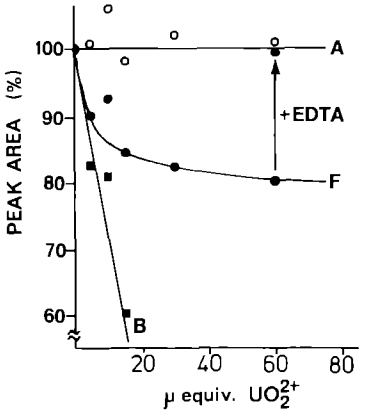

Fig. 4. Peak area of A (orthophosphate), B (phosphomannan) and F (polyphosphate) as a function of the amount of UO_2^{2+} added. The results are expressed in % of the initial value. Arrow: peak area of F after subsequent addition of EDTA.

For peak B this is in accordance with recent investigations, in which this peak could be assigned to phosphomannan, present in the yeast cell wall (Gage *et al.*, personal communication). Peak F stems from the middle phosphate groups of polyphosphates (17,18). Therefore the described observations confirm the localization of a polyphosphate fraction outside the plasma membrane of *Saccharomyces fragilis*, as suggested by previous studies (8-10).

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