Mercury-induced Loss of K^+ from Yeast Cells Investigated by Electron Probe X-ray Microanalysis

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(Received 7 February 1979)

According to Passow & Rothstein (1960), the mercury-induced loss of K^+ from yeast cells is an all-or-none effect. This hypothesis was tested by analysing individual yeast cells by means of energy-dispersive X-ray microanalysis. A dual effect of mercury was observed. The cell population was split into two parts: one part consisted of cells that had suffered a (nearly) complete loss of K^+ – the number of these cells increased with increasing concentrations of $HgCl_2$; the other consisted of cells that had only lost part of their K^+ content – these cells showed a normal distribution around a central value that decreased with increasing concentrations of $HgCl_2$. Our analysis shows that the effect of mercury is more complex than originally suggested and that, in addition to an all-or-none effect, a gradual loss of K^+ occurs.

INTRODUCTION

Passow & Rothstein (1960) have demonstrated that interaction of inorganic mercury with yeast cells results in a leakage of K^+ and anions to the suspending medium. They suggested that this K^+ loss is an all-or-none effect for individual cells, each cell having a certain threshold concentration for mercury at which a complete loss of K^+ occurs. An increase in the $HgCl_2$ concentration results in an increase in the number of cells that suffer a complete loss of K^+ and consequently an increase in the K^+ concentration of the suspending medium.

The elemental content of individual cells can be measured by means of electron probe X-ray microanalysis (Lechene *et al.*, 1977). This technique is based on the detection and recording of X-rays generated within the specimen by the electron beam, each element present in the specimen giving rise to X-rays of a specific energy (Chandler, 1977). Analysis of a considerable number of cells is necessary to determine whether a gradual or an all-or-none effect is involved in K^+ leakage. Roinel (1977) showed the applicability of X-ray microanalysis to problems of this type in a study of the effect of lead on erythrocytes.

In the present study, the effect of inorganic mercury on the K^+ content of yeast cells was examined by X-ray microanalysis. We conclude that both a gradual and an 'all-or-none' response occurred.

METHODS

Incubation. Yeast cells of *Saccharomyces cerevisiae* strain Delft II were starved under aeration for 20 h. After starvation, the cells (2%, w/v) were resuspended in distilled water adjusted to pH 4.0 with HCl. The non-metabolizing yeast cells were incubated for 5 h at 25 °C with $HgCl_2$ at various concentrations. Nitrogen

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was bubbled through the suspension continuously. At appropriate times, 4 ml samples were taken and centrifuged, and the supernatant was decanted. The Na^+ and K^+ concentrations in the supernatant were determined by flame spectrophotometry. The phosphorus content of the supernatant was determined by the method of Fiske & Subbarow (1925).

Preparation of the cells. After 5 h incubation, the cells were washed twice with distilled water (pH 5.6) and resuspended in distilled water. The cell suspension was sprayed through a glass capillary tube (internal diam. about 0.5 mm) on to polished pure carbon plates. To demonstrate that no loss of cell K^+ occurred during washing in distilled water, the supernatant from the washed suspensions was analysed by flame spectrophotometry. The carbon support was exposed to the spray for about 5 s at a distance of 20 cm. The cells were air-dried on the support and then stored under vacuum at room temperature. The specimen was coated with a carbon layer to improve conductivity and, to prevent rehydration, it was kept under vacuum until analysis.

Electron probe X-ray microanalysis. Analysis was performed with an EDAX energy-dispersive spectrometer in combination with a Philips PSEM 500 scanning electron microscope. The carbon support was mounted on an aluminium specimen holder. The carbon plate had a larger diameter than the aluminium holder so that the production of stray X-rays from the aluminium holder was avoided. Analysis was carried out at an accelerating voltage of 25 kV and a spot size of 0.25 μm . The specimen was tilted 20° with respect to the electron beam. Calculations on the X-ray spectrum were carried out with the EDIT 7 EM software system.

RESULTS

Yeast cells used in this study contained about 250 mmol K^+ per l cell water. The yeast cell is relatively impermeable to cations. If cells are suspended in distilled water, only a very small leakage of K^+ occurs: in resting cells, a steady state concentration gradient of about 1750:1 is maintained (see Fig. 1).

Passow & Rothstein (1960) showed that inorganic mercury caused a breakdown in the permeability barrier to K^+ : thus incubation of non-metabolizing cells in the presence of HgCl_2 resulted in a large efflux of K^+ from the cells into the medium. Figure 1 shows that HgCl_2 caused a K^+ leakage from yeast cells. The amount of K^+ that leaked out of the cells after 5 h incubation was dependent on the HgCl_2 concentration; at HgCl_2 concentrations above 0.4 mM, almost all the cell K^+ was lost. The time course of the leakage was also dependent on the HgCl_2 concentration; the half-time of the K^+ efflux decreased from 90 min with 0.11 mM- HgCl_2 to only 10 min with 0.8 mM- HgCl_2 .

Analysis of the supernatants from the washed cell suspensions showed that K^+ loss as a result of washing in distilled water was very small. Furthermore, there was no measurable Na^+ leakage from the cells during the mercury treatment. Phosphorus leakage was found to be negligible; at 0.8 mM- HgCl_2 , only 4% of the total internal phosphorus content leaked out of the cells.

During incubation of yeast cells in HgCl_2 , a pH change of the suspending medium was observed, which was dependent on the HgCl_2 concentration and the incubation time (Fig. 2). A maximum in the pH curves was observed, and this maximum was also dependent on the HgCl_2 concentration.

After 5 h incubation at a given concentration of HgCl_2 , the yeast cells were mounted on a carbon plate by spraying (see Methods); this gave a large number of well separated cells. Scanning electron microscopy of the mercury-treated cells showed no obvious differences between or within cell populations. It should, however, be remarked that we examined carbon-coated specimens only, so that no optimal image was obtained.

The yeast cells were examined by means of electron probe X-ray microanalysis. The carbon plate produced only low background X-ray intensity. X-ray signals from P, S, Cl and K were found only in cells and not in adjacent areas of the support. Typical X-ray spectra of yeast cells incubated in 0.13 mM- HgCl_2 are shown in Fig. 3.

Variations in cell size will be reflected in the absolute intensities of the characteristic K and P signals. Since the electron beam penetrates the yeast cells completely, part of the

Fig. 1

Fig. 2

Fig. 1. Effect of $HgCl_2$ on K^+ efflux from yeast cells suspended in water at an initial pH of 4.0: $\circ$, control; $\bullet$, 0.11 mM- $HgCl_2$; $\square$, 0.13 mM- $HgCl_2$; $\blacksquare$, 0.22 mM- $HgCl_2$; $\triangle$, 0.66 mM- $HgCl_2$; $\blacktriangle$, 0.8 mM- $HgCl_2$.

Fig. 2. Effect of $HgCl_2$ on the pH of the suspending medium. Symbols as in Fig. 1.

0 40SEC 19219INT
VS: 500 HS: 20EV/CH

0 40SEC 21393INT
VS: 500 HS: 20EV/CH

Fig. 3. X-ray spectra from yeast cells treated with 0.13 mM- $HgCl_2$. Characteristic K_α peaks are marked with the corresponding chemical symbols. The spectra were recorded at an accelerating voltage of 12 kV.

observed spectrum is due to the carbon support, which will contribute to the background; hence, the background intensity cannot be used to correct for specimen mass thickness (Hall, 1971). We therefore chose to express the results of the analysis as the ratio between the observed intensities of the K and P signals, assuming that the intensity of the P signal is a good measure of cell volume. This ratio is less sensitive to differences in cell volume and experimental conditions such as beam current than the absolute values of the peak intensities for K and P.

About 150 cells of each population incubated at a given $HgCl_2$ concentration were analysed. The histogram of the probe readings of control cells showed a single population, with a normal distribution around a central value of 1.51 (Fig. 4a). Treatment of the yeast cells with $HgCl_2$ appeared to have a dual effect. After treatment with 0.1 to 0.2 mM- $HgCl_2$, the cell population was split in two parts; cells of one part lost some K^+ , while the others lost almost all their K^+ (Fig. 4b, c, d). At concentrations of 0.22 mM- $HgCl_2$ and above, all cells had lost virtually all their K^+ .

Fig. 4. Distribution of the K/P ratio in a population of yeast cells treated with various concentrations of HgCl_2 : (a) control; (b) 0.11 mM- HgCl_2 ; (c) 0.13 mM- HgCl_2 ; (d) 0.165 mM- HgCl_2 .

Fig. 5

Fig. 6

Fig. 5. Effect of HgCl_2 on K^+ efflux from yeast cells suspended in water ($\circ$ and $\square$) or 10 mM-NaCl ($\bullet$ and $\blacksquare$): $\circ$ and $\bullet$, 0.055 mM- HgCl_2 ; $\square$ and $\blacksquare$, 0.165 mM- HgCl_2 .

Fig. 6. Distribution of the K/P ratio in a population of yeast cells treated with 0.11 mM- HgCl_2 in 10 mM-NaCl.

The K^+ leakage was stimulated in the presence of Na^+ or Mg^{2+} . However, the stimulation of the mercury-induced K^+ efflux by these cations was in contrast with the finding that resting cells lost less K^+ during washing in 10 mM-NaCl or MgCl_2 at pH 4.0 than in distilled water at pH 4.0. K^+ efflux in both 10 mM-NaCl and distilled water (pH 4.0) is shown in Fig. 5. In a parallel experiment it was found that the maximum pH value of the supernatant attained during incubation in 10 mM-NaCl was lower than that in distilled water. The results of the analysis of cells treated with 0.11 mM- HgCl_2 in 10 mM-NaCl (Fig. 6) again indicate two populations. However, compared with the cells treated with the same HgCl_2 concentration in water, the mean K/P ratio in that part of the cell population that had lost some K^+ was decreased, whereas the number of cells that had lost all K^+ was increased.

In Fig. 7, the final K^+ concentration in the incubation medium, as measured by flame

Fig. 7. (a) Effect of $HgCl_2$ on the final K^+ loss after 5 h incubation at an initial pH of 4.0: $\circ$, water; $\bullet$, 10 mM-NaCl added; $\times$, 45 mM-NaCl added.
 (b) Effect of $HgCl_2$ on the mean K/P ratio in a population of yeast cells after 5 h incubation, as measured by electron probe X-ray microanalysis: $\circ$, water; $\bullet$, 10 mM-NaCl added.

spectrophotometry, is plotted as a function of the $HgCl_2$ concentration. The loss of K^+ as calculated from the microprobe analysis of the individual cells is also shown. These observations are in good agreement with each other.

We also tested the thiol reagent *N*-ethylmaleimide and some metal cations for their ability to induce K^+ efflux from yeast cells. *N*-Ethylmaleimide was not very effective; a concentration of 2 mM was needed to induce a loss of 20% of the total K^+ content of the cells. Cu^{2+} was nearly as effective as Hg^{2+} ; after 4 h incubation, 0.06 mM- $CuCl_2$ caused a K^+ loss of about 90% of that caused by the same concentration of $HgCl_2$. Zn^{2+} was much less effective; 5.5 mM- $ZnCl_2$ was needed to obtain a K^+ loss comparable to that caused by 0.11 mM- $HgCl_2$. $CdCl_2$ did not cause any appreciable leakage of K^+ .

DISCUSSION

The method of preparation of yeast cells for microanalysis used in this study permits the analysis of a large number of cells. Air-drying appears to be an adequate method for retaining K^+ in the cells, as was shown by Chandler & Battersby (1976) for spermatozoa and by Lechene *et al.* (1977) for erythrocytes.

The results of our analysis (Fig. 4) show that two effects of mercury can be distinguished. At first, each cell in the population seems to respond in a graded fashion to increasing concentrations of $HgCl_2$, but, when a certain threshold is attained, the cell loses all internal K^+ . Thus our findings are in agreement with the suggestion of Passow & Rothstein (1960) that with increasing concentrations of $HgCl_2$ an increasing number of yeast cells lose all internal K^+ . In contrast with their suggestion, however, we found that K^+ loss is not only an all-or-none process, but that in addition a gradual loss occurs that is related to the mercury concentration. The mechanism of mercury-induced K^+ loss from yeast cells appears to be more complex than the lead-induced K^+ loss from erythrocytes, studied by Roinel (1977) with X-ray microanalysis; in that case, a simple all-or-none response was observed.

In order to maintain electroneutrality, the efflux of K^+ will be accompanied by an influx

of cations and/or an efflux of cell anions. The pH change of the medium during incubation with HgCl_2 (Fig. 2) suggests a concomitant proton influx. However, it appears that mercury induces not just a simple stoicheometric $\text{K}^+ - \text{H}^+$ exchange, but much more complex ion-fluxes. The form of the pH curves compared with the K^+ efflux curves (Figs 1, 2) suggests an additional anion efflux, because H^+ influx is insufficient to account quantitatively for the charge balance. Leakage of organic acids from the cells could explain the decrease in pH at higher mercury concentrations. In addition, the experiments showing an increased efflux of K^+ in a medium containing NaCl (see Fig. 5) suggest the possibility of an exchange of K^+ with cations from the medium (in this case Na^+). Other interactions of mercury with the yeast cell may also occur, e.g. reaction with SH groups, leading to production of protons. The initial decrease in net H^+ influx observed at low mercury concentrations might be related to such interactions.

The biochemical action of heavy metals has been extensively reviewed (Passow *et al.*, 1961; Vallee & Ulmer, 1972). Mercury has an especially strong affinity for thiol groups, copper is also a thiol reagent, while zinc and cadmium have a high affinity for the imidazole group (Boyer, 1959; Gurd, 1954). *N*-Ethylmaleimide is a thiol reagent with a much lower specificity.

Passow & Rothstein (1960) suggested that inorganic mercury interacted with SH groups of proteins in the yeast cell membrane that are involved in maintaining the permeability barrier for K^+ . Shieh & Barber (1973) found that HgCl_2 above a certain concentration caused a net efflux of K^+ in *Chlorella*. They suggested that mercury may produce conformational changes in the membrane and in some way affect a macromolecular carrier. Our results also suggest that the increase in K^+ permeability is associated with the reaction of mercury with SH groups within the yeast cell membrane, but it is unclear how the gradual and total K^+ loss, respectively, are related to the interaction of mercury with the yeast cell.

We are grateful to Dr A. M. Stadhouders, Department of Submicroscopic Morphology, University of Nijmegen, for putting the electron microscope at our disposal. We also wish to thank Dr G. W. F. H. Borst-Pauwels for valuable criticism. This study was supported in part by a grant from the Netherlands Organisation for the Advancement of Pure Research (Z.W.O.) through the Netherlands Foundation for Biophysics. The yeast was kindly supplied by Gist-Brocades at Delft.

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