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Interaction of *Saccharomyces cerevisiae* with gold: toxicity and accumulation

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Abstract

This paper examines the effects of ionic gold on *Saccharomyces cerevisiae*, as determined by long-term (growth in gold-containing media) and short-term interactions (H^+ efflux activity). An increasing gold concentration inhibited growth and at <0.2 mM Au, growth was not observed. Transmission electron microscopy revealed no differences in ultrastructure but fine electron dense particles were observed in unstained preparations from gold-containing medium. After glucose addition (to 10mM) to starved suspensions of *S. cerevisiae*, glucose-dependent reduction of external pH occurred as the cells extruded protons. In the presence of increasing gold concentrations, the lag time before proton extrusion did not change but the rate and duration decreased significantly with a marked influence on proton efflux rate being observed at ≤ 10 μM. Extension of preincubation time of yeast cells in gold-containing medium resulted in a decreasing proton efflux rate and colloidal phase formation in the cell suspensions, the time between gold addition and the beginning of colloidal phase formation depending on the gold concentration used. Both Ca and Mg enhanced the inhibitory effect of gold on the yeast cells with Ca showing a stronger inhibitory effect than Mg.

Introduction

Gold is a widespread element comprising from 10^{-9}–10^{-8} of the absolute mass of the earth (Sobolevsky 1970). The content of sea water is approximately 4 ng Au l^{-1} (Horn 1972), mostly as $[AuCl_2]^{-1}$ (Peshchevitskiy *et al.* 1965; Horn 1972) but can also be present in colloidal, ionic-molecular, and complex-molecular forms (Goleva *et al.* 1970; Nechayev & Zvonarev 1983) and in three possible oxidative states – Au(0), Au(I) and Au(III) (Busev & Ivanov 1973). Terrestrial and aquatic microorganisms, which play important roles in the biogeochemical cycling of metals, also take part in geochemical transformations of Au (Minyeyev 1974; Olson 1994; Savvaidis *et al.* 1998) and have been used in the biological treatment of gold-containing ores (Lawrence 1990).

Gold is a biologically inessential metal and in the ionic state, is a strong oxidizing agent and displays some bactericidal properties (Sadler 1976). Some bacterial strains can introduce Au-binding materials into the medium as apparent protective agents (Higham *et al.* 1986) but the most widespread and gratuitous form of protection appears to be the reduction of ionic gold to the elemental state (Korobushkina *et al.* 1989) or complexation with organic compounds, e.g. amino acids (Chernyak & Shestopalova 1976; Korobushkina *et al.* 1974); and cyanides (Smith & Hunt 1985). These processes occur at comparatively high gold concentrations where the influence of gold on living microbial cells is inhibitory (Pivovarova *et al.* 1986; Korobushkina *et al.* 1987, 1991). From a biotechnological perspective, the most attractive aspect of the interaction of microorganisms with gold is related to the

biosorptive properties of microbial biomass (Greene *et al.* 1986; Hosea *et al.* 1986; Gadd 1988; Karamushka *et al.* 1990; Kuyucak & Volesky 1989a,b,c) since many microorganisms possess high gold sorptional capacities (Kuyucak & Volesky 1989a–c). In addition, certain bacteria (Karamushka *et al.* 1990; Ulberg *et al.* 1990, 1992) and microalgae (Karamushka *et al.* 1990; Ovcharenko *et al.* 1991) appear capable of concentrating gold both in soluble and insoluble (colloidal) forms by energy-dependent mechanisms. For example, it has been shown that gold can form finely dispersed intracellular articles in *Candida utilis* (Biryuzova *et al.* 1987). In spite of this, basic information about mechanism(s) of microbial gold transformations and toxicity, particularly in yeasts, is still lacking. The objective of this particular study therefore was to examine the effects of ionic gold on *Saccharomyces cerevisiae*, as determined by long-term (growth on gold-containing media) and short-term (H^+ efflux activity) interactions.

Materials and methods

Organism and growth conditions

Saccharomyces cerevisiae X2180-1B was used in these studies. Cultures were maintained on MYGP agar comprising (gl^{-1}): malt extract (Lab M), 3.0; yeast extract (Lab M), 3.0; D-glucose, 10.0; neutralized bacteriological peptone (Lab M) 5.0; agar (Lab M No. 2), 15.0. For experimental purposes, cultures were grown in liquid medium comprising (gl^{-1}): KH_2PO_4, 2.72; $K_2HPO_4 \cdot 3H_2O$, 5.22; $(NH_4)_2SO_4$, 2.0; $MgSO_4 \cdot 7H_2O$, 0.012; $CuSO_4 \cdot 5H_2O$, 0.0004; $FeSO_4 \cdot 7H_2O$, 0.0022; $ZnSO_4 \cdot 7H_2O$, 0.004; $MnSO_4 \cdot 4H_2O$, 0.004; D-glucose, 20.0; yeast extract (Difco), 1.0 at 25 °C on an orbital shaker (100 rpm). Experimental cultures (initial O.D. at 550 nm approx. 0.1) were prepared from 48 h starter cultures grown from loop inoculation from MYGP agar. For preparation of cell suspensions, cultures grown for 40–48 h were centrifuged (1200 $\times g$, 10 min), washed twice with and finally suspended in 5 mM piperazine-N,N′-bis[2-ethanesulphonic acid] (PIPES) buffer, adjusted with tetramethylammonium hydroxide pentahydrate to pH 6.5, and stored at 4 °C overnight. Cell numbers were determined using a modified Fuchs-Rosenthal haemocytometer after appropriate dilution with distilled water. To examine the influence of gold on growth of *S. cerevisiae*, cells from a 24 h culture were inoculated into the growth medium containing gold added in the form of tetrachloroaurate at different concentrations 12 h before inoculation.

Assay of proton efflux

The rate of proton efflux was measured by recording pH changes in diluted cell suspensions (Serrano 1980; Gadd *et al.* 1986; White & Gadd 1987; Karamushka & Gadd 1994). 100 μl aliquots of a cell suspension (30×10^8 cells ml^{-1}) were diluted in a total volume of 10 ml 5 mM PIPES buffer, pH 6.5, in a water-jacketed vessel at 25 °C and stirred using a magnetic stirrer. Cells were allowed to equilibrate prior to additions of glucose (to a final concentration of 10 mM), Au, Ca, or Mg. Unless specified elsewhere, Au was added 5 min before glucose addition; Ca and Mg were added 30 s before Au addition. The pH of the cell suspension was measured continuously using a Russell combination pH electrode with gel electrolyte (Russell pH Ltd, Auchtermuchty, Fife, UK) attached to a Kent/EIL 7055 pH meter (Eil Analytical Instruments, Chertsey, Surrey, UK) and recorded continuously using a Servoscribe potentiometric chart recorder. Continuous measurements were standardized by known additions. All incubations were carried out at 25 °C. Throughout the paper typical results are presented, although each experiment was carried out at least in duplicate.

Chemicals

All chemicals used were of analytical grade. Ca and Mg were used as chlorides, gold was supplied as hydrogen tetrachloroaurate trihydrate (Aldrich).

Results

The influence of gold on growth of Saccharomyces cerevisiae

The addition of low concentrations of hydrogen tetrachloroaurate trihydrate to growth medium resulted in the formation of a dispersed phase over 10–12 h incubation. The more gold that was added to the growth medium, the higher was the intensity of colloid formation. The influence of these metal concentrations on the growth of *S. cerevisiae* is shown in Figure 1. As can be seen, an increasing gold concentration caused a strong inhibitory effect and at $\geq$0.2 mM Au, growth was not observed. Electron microscopic examination of *S. cerevisiae* grown in medium with or without gold showed no apparent differences in ultrastructure, even

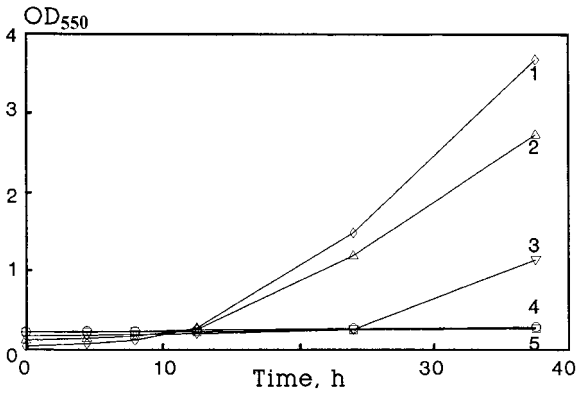

Figure 1. Influence on gold concentration on growth of *S. cerevisiae*. The growth medium contained 0 (1), 100 μM (2), 150 μM (3), 200 μM (4), or 250 μM (5) $HAuCl_4$. Typical results are shown from one of several experiments.

at relatively high gold concentrations (0.1 mM). However, several electron-dense areas containing fine particles were observed in unstained transmission electron micrographs of cells from gold-containing media in contrast to gold-free cultures (results not shown).

Influence of gold on H^+ efflux from Saccharomyces cerevisiae

At a constant cell concentration in suspension, the initial H^+ efflux rate depends on several physicochemical properties of the surrounding medium, including the glucose concentration. Despite the high initial glucose concentration in the growth medium (50 mM), the rate of proton efflux by *S. cerevisiae* attained a maximum value at a glucose concentration of approximately 5 mM and remained at approximately the same level up to a glucose concentration of 30 mM (data not shown). Therefore, as well as taking into account that glucose can be an effective reducer of trivalent gold, glucose was used at a final concentration of 10 mM in all the experiments on proton efflux.

Initial addition of gold to the incubation medium caused acidification of the solution since dissociation and hydrolysis of tetrachloroaurate occurred (Figure 2). After glucose addition to a starved suspension of *S. cerevisiae*, a glucose-dependent reduction of external pH was observed as the cells extruded protons (curve 1). This process can be characterized by such parameters as lag time (the time period between glucose addition and the start of pH reduction), efflux rate (the change of pH per unit time) and duration (the time period between glucose addition and achievement of a new state of equilibrium). The initial rate (R_0) of this process (measured over the time period between 5 and 15 min after glucose addition) was 11.3 ± 0.5 nmol H^+ min^{-1} $(10^7$ cells$)^{-1}$. In the presence of increasing concentrations of gold, the lag time before proton extrusion occurred did not change but the rate of this process and its duration decreased significantly. A marked influence of gold on proton efflux rate was observed at a gold concentration of 10 μM and higher. The time of preincubation of yeast cells in gold-containing medium was also an important determinant of the medium acidification process and two particular features can be emphasised. Firstly, extension of the preincubation time at least up to 1 h resulted in a decreasing proton efflux rate by *S. cerevisiae* and, secondly, colloidal phase formation occurred in the cell suspensions (Figures 3 and 4). The time between gold addition to the cell suspension and the beginning of colloidal phase formation depended on the gold concentration used: this time was less when the gold concentration used was higher. Although an increase in the gold concentration resulted in the inhibition

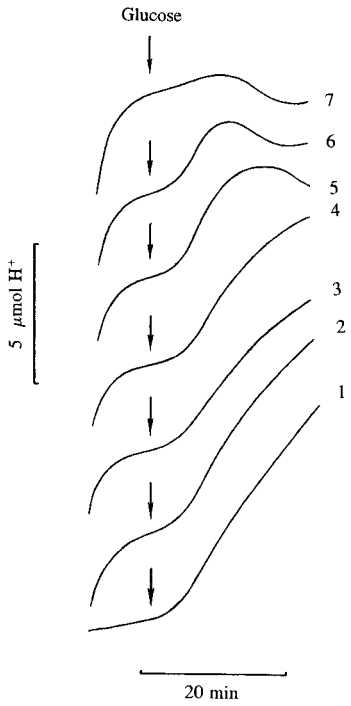

Figure 2. Influence of $HAuCl_4$ on H^+ efflux by *S. cerevisiae*. Cells were suspended in 5 mM PIPES, pH 6.5 (1–7) as well as 50 μM (2), 150 μM (3), 200 μM (4), 300 μM (6) and 400 μM (7) $HAuCl_4$. Glucose was added at the point indicated to 10 mM final concentration. Typical traces are shown from one of at least three experiments.

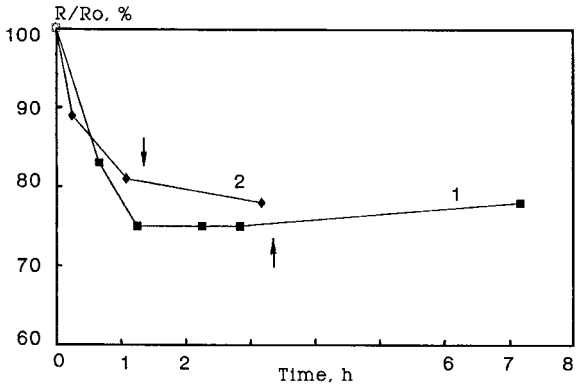

Figure 3. Influence of preincubation time before glucose addition on H^+ efflux rate of *S. cerevisiae* in the presence of (1) 50 μM and (2) 100 μM $HAuCl_4$. Arrows indicate the apparent onset of colloidal gold formation in incubation medium. Typical results are shown from one of at least three experiments.

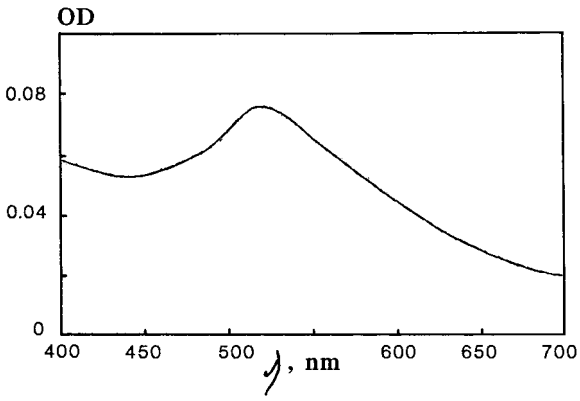

Figure 4. Absorbance spectrum of incubation medium showing colloidal gold formation in a cell suspension of *S. cerevisiae* containing 50 μM $HAuCl_4$. The incubation time was approximately 2 h, the cell concentration was 1.6×10^7 ml^{-1} in 5 mM PIPES buffer, pH 6.5, in a total volume of 10 ml. Typical results are shown from one of at least three experiments.

of proton efflux, within a certain range of concentration, a decreasing pH was initially observed which was then followed by a medium pH increase. At comparatively high gold concentrations (0.4 mM and more), the ability of the cells to extrude protons was fully blocked.

Influence of Ca and Mg on H^+ efflux from Saccharomyces cerevisiae *in the presence of gold*

It has been shown that Ca^{2+} and Mg^{2+} may exhibit protective properties against the inhibitory influence of some heavy metals (e.g. Cu, Mn and Co) towards *S. cerevisiae* (Karamushka & Gadd 1994; Karamushka *et al.* 1996). Another effect was revealed when

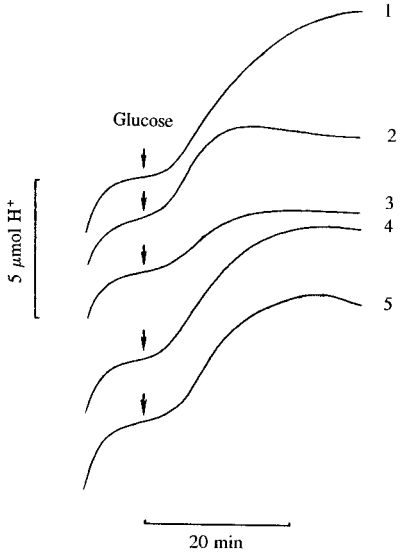

Figure 5. Influence of $HAuCl_4$, Ca and Mg on H^+ efflux from *S. cerevisiae*. The suspension medium was 5 mM PIPES, pH 6.5, containing 200 μM $HAuCl_4$ (1–5) as well as 0.5 mM (2) and 0.1 mM (4) $MgCl_2$, or 0.5 mM (3) and 0.1 mM (5) $CaCl_2$. Typical traces are shown from one of at least three experiments.

ionic gold was used. The curves reflecting changes in medium pH in the presence of gold and different concentrations of both mg and Ca are presented in Figures 5 and 6. Qualitative comparison of experimental data obtained at the gold concentrations of 0.2 mM (Figure 5) and 0.3 mM (Figure 6) reveal that for tetrachloroaurate, both Ca (Figure 5, curves 3–5; Figure 6, curves 2–5) and Mg (Figure 5, curves 2, 4; Figure 6, curves 6–8) intensified the inhibitory effect of gold on the yeast cells. Moreover, in contrast to the metals used in ionic form in previous studies (Zn, Cu, Mn, Co) (Karamushka & Gadd 1994), Ca showed stronger inhibitory properties than Mg. The sensitivity of the cells to Ca was observed at a lower concentration in comparison with Mg (Figure 6, curves 2 and 6). At the same concentrations, Mg exhibited a less significant influence (Figure 5, curves 2, 3, 4 and 5).

Discussion

Gold belongs to a group of widespread elements but its overall concentration in the biosphere is so low that any significant influence in this metal on microorganisms may be evident only in specific local regions stressed by natural or anthropogenic sources. Due to high oxidative properties and the ability to complex with many organic compounds, gold is usually present

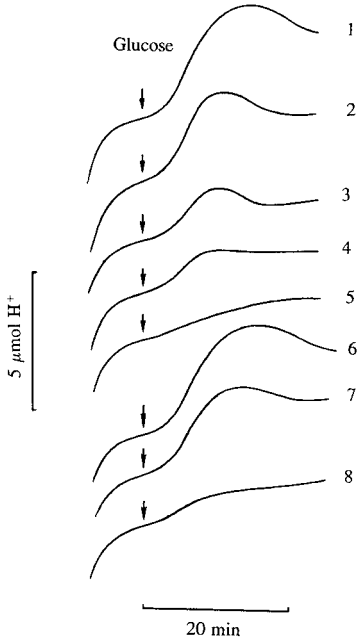

Figure 6. Influence of $HAuCl_4$, Ca and Mg on H^+ efflux from *S. cerevisiae*. The suspension medium was 5 mM PIPES, pH 6.5, containing 300 μM $HAuCl_4$ (1–8) as well as 5 μM, 50 μM, 100 μM and 500 μM $CaCl_2$ (2, 3, 4 and 5 respectively) and 25 μM, 100 μM and 500 μM $MgCl_2$ (6, 7 and 8 respectively). Typical traces are shown from one of at least three experiments.

in natural conditions in the form of soluble metalloorganic compounds or insoluble metal particles (Peshchevitskiy *et al.* 1965; Goleva *et al.* 1970). Despite this, it is desirable to develop an understanding of those mechanisms which underlie the interactions between microbial cells and 'exotic' metals like gold because these fundamental processes are the basis for their application in such important spheres as metallodrug pharmacology, mineral biotechnology and environmental protection (Savvaidis *et al.* 1998).

From the data obtained, it appears that non-adapted yeasts are sensitive to ionic gold but quite insensitive to reduced gold. Ionic gold was added to the culture medium approximately 10 h before the cells were inoculated and after that time, intensive colloidal phase formation was observed. Undoubtedly, the formation of organo-gold compounds took place simultaneously because the yeast extract used as a necessary component of the culture medium, contains many different organic compounds. Since colloidal gold generally is quite inert and evidently does not possess any marked influence on microbial activities (Bagnyuk *et al.* 1990; Karamushka *et al.* 1991), it was likely that growth inhibition was caused by soluble gold complexes. This could be the reason for the slightly increasing proton efflux rate when the preincubation time with gold was increased (Figure 3, curve 1): due to colloidal gold formation, the concentration of effective soluble ionic forms decreased.

The toxic influence of ionic gold was demonstrated in short-term experiments on the measurement of proton efflux by *S. cerevisiae*. Two peculiarities of this process have been revealed in comparison with the influence of other toxic metals on H^+ efflux (Karamushka & Gadd 1994). An increasing ionic gold concentration resulted in a decrease of the rate of H^+ efflux and the duration of this process as occurs when other metals (Cu, Co, Mn) were used (Karamushka & Gadd 1994). However, the second peculiarity of the process investigated consists of the differing influence of Mg and Ca on the effect of ionic gold where it was found that, within a certain range of concentrations, both Mg and Ca possessed an inhibitory effect which increased with increasing concentration. It should be emphasized that no conditions were found which revealed any protective properties of Mg and Ca in the presence of ionic gold as was shown during the examination of proton efflux by *S. cerevisiae* in the presence of essential and inessential metals (Karamushka & Gadd 1994). The cause of this difference is unclear but it is pertinent to indicate that gold, in spite of its addition to the medium as tetrachloroaurate, easily changes its form and may be present in numerous states both in valency (III, I, 0) and in ligand type. Therefore, gold may have different influences on cells, e.g., Au(III) possesses strong oxidative properties (Busev & Ivanov 1973) while Au(0) may be a catalyst (Bagnyuk *et al.* 1990). Such changes in speciation undoubtedly affect gold sorption and accumulation by the yeast cells. The presence of colloidal gold in the medium prevented separation of cells from unbound gold as cells sedimented together with colloidal gold particles during centrifugation, and therefore discerning the quantity inside the cells and associated with cell surfaces. However, no obvious gold deposits were observed in transmission electron micrographs of cells grown in the presence of gold, in contrast to numerous gold particles located outside the cells. This appears to be in contrast with *Candida utilis* which accumulated gold added to the growth medium in both ionic and elemental forms but localized it in different organelles as well as on the cell surface (Korobushkina *et al.* 1987; Biryuzova *et al.* 1987).

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