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Time dependent formation of gold nanoparticles in yeast cells: A comparative study

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ABSTRACT

Two different strains of yeast, *Saccharomyces cerevisiae*, AP22 and CCFY-100 were studied for bioaccumulation of gold in the form of $H^{198}AuCl_4$. Thin sectioning and subsequent study by transmission electron microscopy (TEM) reveals that Au^{3+} was *in situ* reduced to Au(0) and nano sized gold particles were formed inside the cell. Very low dose γ-energy being responsible for reduction of cationic gold in the polymeric cytoplasm matrix. The formation and entry of gold nanoparticles in the yeast cells were studied as a function of time at certain intervals starting from 15 min to 72 h. The gold nanoparticles gradually moved inward as a function of time from cell wall to cytoplasm to nucleus and finally accumulated in the nucleolus of the cell. TEM image of budding yeast shows that gold nanoparticles are not transferred to the new generation yeast cells.

1. Introduction

Biological substrates have shown promising abilities for interaction with metal ions [1–3]. Proteins and carbohydrates of cell wall provide good sites for metal binding [4]. They appear to be the safer alternatives towards designing greener technology for the future [5]. Among various eukaryotic organisms yeasts play an important role as a model of eukaryotic cells for biochemical and physiochemical experiments. For example, the effects of some heavy metals on the growth of some soil-yeasts were studied [6]. As a useful means of bioremediation of environmental chromium contamination, yeasts were used to treat Cr containing effluents in order to remove toxic compounds from waters and soils [7]. Earlier, we have also reported extraction of ^{152}Eu, a long-lived fission product, by yeast cells, *Saccharomyces* [8]. The dry waste biomass of powder of *Saccharomyces cerevisiae* obtained from beer fermentation was studied for Au^{3+} biosorption [9].

Synthesis of gold nanoparticles from different biological agents like bacteria, fungi and plant extracts have been reported earlier, which have potential applications in hyperthermia in cancer cells [10]. Recently, the use of precious metal gold in nanotechnology has increased in leaps and bounds due to its wide application in electronics, chemistry, and engineering owing to their specific optical and electrical properties. Moreover, gold nanoparticles are promising in the field of clinical sciences, especially for in vivo application. The intracellular synthesis of gold nanoparticles of various morphologies and sizes in two fungal cultures, *V. luteoalbum* and Isolate 6-3 has been documented earlier [11]. The size of nanoparticles can be manipulated by controlling parameters such as pH, temperature, gold concentration and exposure time to gold solution. On the other hand, the effects of ionic gold on *S. cerevisiae*, was determined by long-term and short-term interactions [12]. The addition of low concentrations of gold as tetrachloroaurate salt to growth medium resulted in the formation of a dispersed phase over 10–12 h incubation. Transmission electron microscopy revealed no differences in ultrastructure. No gold deposits were observed in transmission electron micrographs of cells grown in presence of gold, in contrast to numerous gold particles located outside the cell.

From our earlier work on synthesis of gold nanoparticles, we have observed that minuscule amount of *in situ* radioactivity from ^{198}Au radioisotope ($T_{1/2}$ = 2.69 d) can induce radiolysis in polymer matrix (polyethylene glycol, PEG) and in turn reduce Au(III) to Au(0) [13]. Similarly, minute amount of *in situ* radioactivity from ^{198}Au radioisotope is also capable of synthesizing Au–Pd bimetallic nanoparticles in a PEG matrix [14]. Biological cells are composed of various polymeric substances, e.g., cell wall of yeast is made of 30–60% polysaccharides (beta-glucan and mannan sugar polymers), 15–30% proteins, 5–20% lipids and a small amount of chitin [15]. Considering this fact, we thought that such media may also

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provide suitable condition for reduction of Au^{3+} using the great power of minuscule *in situ* radiation.

In the present work, we report the formation of gold nanoparticles in yeast cells from $H^{198}AuCl_4$. The nature and site of gold nanoparticles formed inside the cells were studied using TEM after cutting thin sections. To the best of our knowledge, for the first time migration of gold nanoparticles was studied inside the cell as a function of time using TEM.

2. Experimental

The radioisotope, ^{198}Au was supplied by Board of Radiation and Isotope Technology (BRIT), Mumbai, India. Yeast extract and bactopeptone used for the preparation of media were of HI Media, agar and dextrose used were of SISCO Research Laboratories, India. All other chemicals were of AR grade. The ^{198}Au was measured by studying the area under 411 keV characteristic photopeak of ^{198}Au by an HPGe detector having a resolution of 2.0 keV at 1.33 MeV was used. In our experiment, 0.01 mCi ^{198}Au was mixed with the stable gold solution (5 mM). The minute amount of *in situ* radioactivity reduced the bulk gold in yeast cells to form nanoparticles. The number of atoms of radioactive ^{198}Au is only 1.2×10^{11} (in 0.01 mCi). The number of stable gold atoms in 5 mM, 100 μL gold solution is 3.0×10^{17}. Thus the ratio of stable ^{197}Au: radioactive ^{198}Au is 2.5×10^{6}. The strains used for accumulation study were AP22 (*MATα leu2 his3 trp1 ura3*) had a background from G. R. Finks laboratory and CCFY 100 (*MAT*a *leu2 his3 ade2 ura3 trp1 can1*) had W303 strain background.

2.1. Preparation of media

A solution containing yeast extract (0.5%), bactopeptone (1%), dextrose (1%), agar (1.5%) was autoclaved to sterilize, allowed to cool for 10 min and poured in a sterile plate in sterile condition. The media was allowed to stand until it set into gel. Different strains of yeast were allowed to grow on this media and kept in 30 °C incubator for 24 h to allow the yeast cells grow.

2.2. Bioaccumulation study at different pH

The yeast cells grown on this medium were suspended in 5 mL water and shaken to make a uniform mixture. For the study of the variation in metal uptake with pH, 3 mL of the solution of required pH, adjusted with dil. HCl or NaOH were shaken for 10 min with 100 μL of yeast suspension and 100 μL (∼1 μCi) of $H^{198}AuCl_4$ tracer solution. The activity of the radioactive solution was measured (C_1) for 10 min in a fixed geometry before mixing to yeast suspension. The resulting mixture was then filtered using a syringe filter (0.45 μm pore diameter) and the filtrate was taken for counting (C_2). The accumulation by yeast cells (A) therefore can be calculated by the following formula:

$$A = \frac{1 - C_2}{C_1} \times 100\%$$

2.3. Study of accumulation kinetics

To determine the rate of accumulation with time, the yeast cells were allowed to remain in contact with $H^{198}AuCl_4$ tracer solution for different intervals of time. The pH was maintained at which maximum accumulation was shown by respective strain as obtained from the previous results.

All sets of data were compared with blank containing gold radiotracer of same volume but without yeast. Appropriate decay corrections have been made for each set of data. The quantitative relation of the number of yeast cells with the accumulation of gold was determined at the best pH condition of gold uptake.

2.4. Accumulation studies with bulk gold

Different concentrations of chloroauric acid solution ($HAuCl_4$) (1–500 mg kg^{-1}) were spiked with radioactive $H^{198}AuCl_4$. 3 mL of this spiked solution was equilibrated with 100 μL of yeast suspension at the pH of their maximum accumulation (pH 3). The resulting mixture was filtered after 15 min using syringe filter and the filtrate was taken for counting. The results were compared with standard $H^{198}AuCl_4$ solution.

2.5. Quantitative relation

The quantitative relation of the number of yeast cells with the accumulation of gold was determined at the best pH condition of gold uptake. The total amount of uptake of gold was studied using different macro amounts of gold solutions of ($HAuCl_4$) spiked with ^{198}Au and the corresponding number of yeast cells was counted using a hemocytometer.

2.6. Study of surface plasmon resonance

The nanogold containing yeast cells were washed thoroughly with 0.5 M phosphate buffer solution and then vortexed with half-volume glass beads in the same buffer medium for mechanically disrupting the cells [16]. The broken cells along with the glass beads were then centrifuged to separate the cell debris at the bottom and the supernatant containing the cytoplasm. The supernatant was taken for UV–visible spectrophotometry to match the surface plasmon resonance of gold nanoparticles. The experiments were repeated at least thrice to check the reproducibility of results.

2.7. Kinetics of gold nanoparticles inside the yeast cells

Freshly growing yeast cells were suspended in 5 mL water, vortexed thoroughly and then 0.1 mL of the solution was added to an HCl solution (pH 3) containing 0.1 mL of $H^{198}AuCl_4$. After certain intervals of addition 0.1 mL of this solution was added to 1 mL of glutaraldehyde (6% in 0.25 M phosphate buffer medium) for fixation. Cells fixed at intervals of 15 min, 1 h, 24 h and 72 h in glutaraldehyde were further treated for thin sectioning and subsequent transmission electron microscopic studies were carried out. These fixed cells in gold accumulated condition were stored under refrigeration for three months to allow complete decay of ^{198}Au before thin sectioning to avoid radioactive contamination in the instruments involved in thin sectioning and electron microscopy.

2.8. Sample preparation for thin sectioning of yeast cells followed by TEM

Before cutting thin sections, the cells need to be fixed. For primary fixation, the gold accumulated yeast cells were suspended in 1 mL of glutaraldehyde (6% in 0.25 M phosphate buffer medium), centrifuged and supernatant discarded. The cells were resuspended in the same above solution and kept at 4 °C for approximately 3 h. The cells were then washed twice using the same buffer and then resuspended in 2% OsO_4 and kept overnight at room temperature for post fixation. The solution was then spun down in microcentrifuge, supernatant discarded and the pellet was hardened in 1% molten agar. The pellet was cut into small pieces (∼1 mm cube) and then dehydration was done using graded alcohol 70%, 80%, 90%, 95% and 100%). The cells were then embedded with equal volume of 100% absolute alcohol and Spurr resin (a low-viscosity