Accumulation of Elemental Gold on the Alga *Chlorella vulgaris*

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Abstract

The accumulation of Au(0) by lyophilized preparations of the alga *Chlorella vulgaris* has been investigated. Gold is bound to the algae upon suspending dried algal cells in solutions containing hydrogen tetrachloroaurate (III). Relative amounts of ionic and atomic algal-bound gold were determined by thiourea extraction. It was found that the amount of algal-bound atomic gold produced from ionic gold increased with time. The effect of algal-bound gold concentration on the rate and extent of gold reduction was observed. It is suggested that at least three different classes of sites are available for gold binding and reduction. The effect of Au(0) accumulation on the binding ability of gold-bound algae was also investigated, and an apparent enhancement of gold binding ability is reported.

Introduction

The interaction of gold with biological systems and biologically derived molecules has been a topic of interest in widely divergent fields of study. The development of gold containing drugs for the treatment of arthritis has stimulated research into the reactions and mechanisms involved in gold transport and deposition in blood and tissues [1–3]. Interactions of Au(III) and Au(I) with biologically significant molecules *in vitro* have also been investigated [4–11]. These studies reveal that the predominant oxidation state of gold in biological systems is +1, that Au(III) is reduced to Au(I) by several biomolecules, and that under certain conditions in prepared solutions, biomolecules can reduce Au(I) to atomic gold.

Interactions of gold with microorganisms have also been investigated. Zumberg, Sigleo and Nagy [12] and Hallbauer [13] have produced evidence implicating the involvement of Precambrian algal blooms and bacteria in the formation of certain gold deposits in South Africa. More recently, Dissanayake and Kritsotakis [14] reported gold accumulations as high as 1.1 ppm in algal mats near the coast of Sri Lanka.

Previously reported results from our laboratory [15] have demonstrated the ability of the alga *Chlorella vulgaris* to adsorb gold ions from aqueous solutions under a variety of conditions, suggesting its potential use as part of a gold recovery system, applicable to natural waters and process streams. These studies also indicated that Au(III) is reduced to Au(I) either concomitantly upon binding to the algae or immediately thereafter, and that under certain conditions, algal-bound Au(I) is further reduced to Au(0). In this paper we investigate the parameters which influence the accumulation of Au(0) on the alga *C. vulgaris*, examining the effect of bound gold concentration on the rate and extent of reduction of algal-bound Au(I). Our results suggest the presence of at least three different classes of gold binding sites on the algal cell. The effect of Au(0) accumulation on additional gold uptake by gold-bound algae is also studied.

Experimental

Gold powder and hydrogen tetrachloroaurate(III) monohydrate were obtained from Strem Chemicals, Inc. Gold standards (1.00 to 5.00 mM) were prepared by dissolving gold powder in aqua regia and diluting appropriately. Experiments were performed using solutions prepared from standardized $HAuCl_4$ stock solutions. Thiourea (99 + %) was obtained from Sigma Chemical Co., and solutions were prepared daily as needed. All other chemicals were reagent grade. Distilled–deionized water was used exclusively in these experiments and all glassware was rinsed with 50% nitric acid prior to use.

Gold analyses were performed using an Instrumentation Laboratory IL457 atomic absorption spectrophotometer with an air–acetylene flame. Electron micrographs were obtained using a Siemens Elmiskop IA.

Procedures regarding the culture and preparation of the alga *Chlorella vulgaris* are reported elsewhere [15]. In order to minimize the effects of extracellular or algal-derived biomolecules that were solubilized when lyophilized algae were suspended in an aqueous

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phase, the algal cells were washed prior to use. This was accomplished by suspending the cells in 0.01 M sulfuric acid, agitating the suspension for a few minutes, centrifuging, and decanting. This procedure was repeated twice. The pH of the algae was then adjusted to the desired value by adding sodium hydroxide or sulfuric acid to the stirred algal suspension.

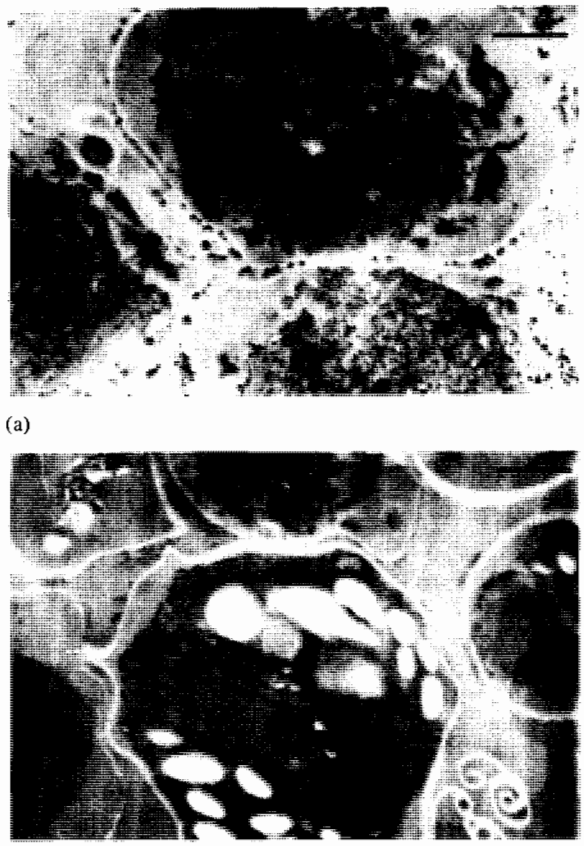
Gold was bound to *C. vulgaris* by suspending the washed algal cells at 2.0 mg/ml in a 0.10 to 0.80 mM solution of $HAuCl_4$ and agitating continuously for 15 minutes. After centrifugation the supernatant was analyzed, and the amount of algal-bound gold determined by difference. The gold-bound algal material was then stored moist in capped centrifuge tubes.

Ionic gold was stripped from the algae by resuspending the cells in 0.10 M thiourea in 0.01 M HCl, agitating the suspension for 15 min, centrifuging, and analyzing the supernatant for desorbed gold. To ascertain the effect of oxygen on thiourea stripping, gold was extracted from the algae both in the presence and absence of oxygen. In these experiments, gold-bound algal cells were resuspended in 0.01 M HCl. One set of samples was then capped with rubber septa and purged with nitrogen for 30 min. A deoxygenated solution of thiourea in 0.01 M HCl was then added by means of a syringe. A thiourea solution equilibrated with air was added to the control samples. The final thiourea concentration in all samples was 0.10 M. After agitation for 15 min, the samples were centrifuged and the supernatant analyzed for gold.

Samples were prepared for electron microscopy in the following manner. *C. vulgaris* was washed with 0.01 M sulfuric acid and divided into two equal portions. One aliquot (gold-free control) received no further treatment. The other sample was equilibrated repeatedly with 0.50 mM $HAuCl_4$ for a total of five, 15 min exposures. The algae were then stored moist in capped centrifuge tubes for 72 h. Samples were then fixed in 2.5% glutaraldehyde for one hour at room temperature, dehydrated in a graded series of acetone solutions, and embedded in Spurr's resin. All samples were viewed unstained.

Results and Discussion

Previously reported experiments [15] have indicated that Au(III) is reduced to Au(I) upon binding to *C. vulgaris* and is retained as a linear algae–Au(I)–chloride complex. Furthermore, under certain conditions, bound Au(I) is slowly reduced to Au(0). These conclusions are based, in part, upon spectroscopic data which demonstrated that only Au(I) can be extracted from algae initially equilibrated with Au(III), and that after an appropriate incubation time, algal-bound gold exhibits a visible absorption spectrum indicating the presence of atomic gold on the algal surface. Atomic gold bound to algal cells is shown in the electron micrograph of Fig. 1a. Gold-free algal cells are shown in Fig. 1b. Algal-bound gold appears in Fig. 1a as numerous dark spots covering the algal cells. Such spots are not seen on the gold-free control. Gold crystals in Fig. 1a are shown by arrows. These crystal shapes are very similar to those reported by Westdorp *et al.* [16] and Komoda [17]. The former observed the formation of tetrahedral crystals upon quenching of heated gold foils while the latter reported the formation of decahedral and icosahedral structures composed of tetrahedral subunits upon crystallization of evaporated gold atoms. It is clear from Fig. 1a that gold accumulation is not restricted to any specific region of the algal cell. Gold deposition seems to occur on interior as well as exterior cell surfaces. One must remember, however, that these algae have been freeze-dried, and damage to cell walls may have occurred. Intact cells may or may not



(a)

(b)

Fig. 1. Electron micrographs of algal-bound gold (a) and gold-free algae (b). Magnification is 9900. Bars represent one micron. Arrows indicate gold crystals.

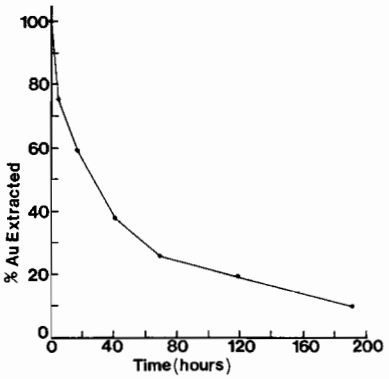


Fig. 2. Amount of algal-bound gold extracted as function of residence time of gold on the algal cells. Algae were saturated by repeated exposure to 0.50 mM $HAuCl_4$ and extracted at pH 2.0 with 0.10 M thiourea after the indicated incubation time.

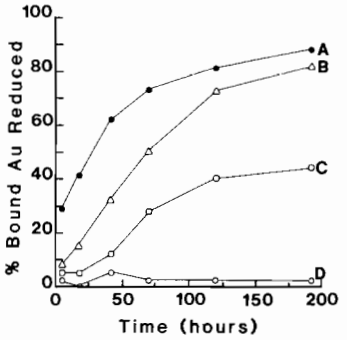


Fig. 3. Rate of reduction of algal-bound gold to Au(0). Initial amounts of algal-bound gold were (a) 0.46 mmol/g dry algae, (b) 0.19 mmol/g dry algae, (c) 0.10 mmol/g dry algae, (d) 0.05 mmol/g dry algae. The percent of bound Au reduced was calculated as (mol Au bound – mol Au extracted ÷ mol Au bound) X 100.

behave in a similar fashion. We are currently investigating this question.

It is well known that thiourea forms soluble complexes with Au(I). It is the relative stability of these complexes which makes thiourea an attractive component in acidic leaching solutions used for the extraction of gold from ores and concentrates [18, 19]. The ability of thiourea to solubilize gold is, however, dependent upon the oxidation state of the gold atoms. In order for the Au(I)–thiourea complex to form, gold must either be present as an ion or be in the presence of an oxidizing agent [18, 20]. In the absence of a suitable oxidant, thiourea will have no effect on elemental gold. While air oxidation of Au(0) in the presence of thiourea can occur, the kinetics are extremely slow [20] so that hours are required for significant amounts of gold to be taken into solution.

This differential rate of solubilization of Au(I) and Au(0) by thiourea provides an indirect method for distinguishing ionic from atomic algal-bound gold. If gold-bound algae are suspended in a 0.10 M solution of thiourea under appropriate conditions, ionic algal-bound gold will be stripped from the algae and taken into solution as the Au(I)–thiourea complex while atomic, algal-bound gold will remain bound to the algal cells. When the algae are removed from suspension, only that gold which was present on the algae in ionic form remains in the supernatant. An application of this technique is demonstrated in Fig. 2. If algal-bound gold is extracted with thiourea immediately after binding, virtually all of the gold is recovered. This indicates that all of the algal-bound gold is still present as ionic gold. If, however, one allows 196 hours to elapse before extracting, only 12% of the gold is recovered. This implies that 88% of the algal-bound gold has been reduced to Au(0) and is no longer extractable under the given conditions. Control experiments (data not shown) indicated that thiourea extraction period of 15 min in the presence of air was not long enough to oxidize atomic gold.

The possible involvement of photochemical reactions in the reduction of algal-bound gold was investigated. After equilibration with a solution of $HAuCl_4$, duplicate algae samples were wrapped in aluminum foil and incubated for 72 h. A second pair of duplicate samples were allowed to incubate for 72 h in room light. All samples were then extracted with thiourea. The results of this experiment (data not shown) indicated that nearly identical amounts of gold are reduced in the presence and absence of light. This suggests that photochemical processes do not play a significant role in this reduction reaction.

Figure 3 illustrates the effect of increasing concentrations of algal-bound gold on the apparent rate and extent of Au(I) reduction. Two points should be noted. First, greater amounts of algal-bound gold result in more rapid gold reduction. Secondly, the extent of gold reduction is also a function of the concentration of algal-bound gold. For example, curve A of Fig. 3 exhibits a much faster rate of reduction than curve C. Curve A also shows a greater degree of reduction (approximately 90%) than curve C (approximately 50%). Curve D illustrates an interesting corollary of the second point. If the algal-bound gold concentration is low enough, (0.05 mmol/g) virtually no gold reduction occurs. These results imply that there are at least two different classes of sites on the algal cell. One class of sites allows the reduction of algal-bound gold while the second class does not. The former sites may be associated with weaker binding since they are most apparent at higher algal-bound gold concentrations (*i.e.*, curves A and B of Fig. 3). The latter sites may be associated with stronger binding since they are most apparent at lower algal-bound gold concentra-

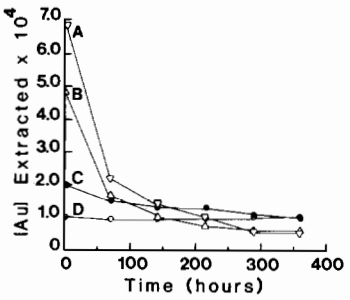


Fig. 4. Amount of algal-bound gold extracted as a function of residence time and concentration of gold on the algal cells. The algal cells were first exposed to gold solutions, washed and incubated for various times after which they were extracted with 0.10 M thiourea at pH 2. Algal-bound gold concentrations were (a) 0.34 mmol/g, (b) 0.19 mmol/g, (c) 0.10 mmol/g, (d) 0.05 mmol/g.

tions (*i.e.*, curve D of Fig. 3). Curve C of Fig. 3 exhibits the behavior expected of an intermediate algal-bound gold concentration, displaying a balance of influence from both classes of sites.

In Fig. 4 the amount of thiourea-extractable gold is shown as a function of time for various concentrations of algal-bound gold. Curve D of this figure again demonstrates the fact that at a concentration of 0.05 mmol/g, algal-bound gold is not reduced. If, however, the algal-bound gold concentration is increased so that Au(0) begins to accumulate at the weaker sites, some of the gold which is bound at the stronger sites becomes reduced. This is apparent from the fact that curves A, B, and C cross curve D in Fig. 4. It is also apparent from Fig. 4 that some ionic algal-bound gold is not reducible even at higher algal-bound gold concentrations. This conclusion rests upon the fact that curves A and B converge and level off before reaching the abscissa. These results imply that there are at least three different classes of sites on the algae. One class, composed of weak-binding sites, provides an environment which permits the facile reduction of bound Au(I) to Au(0). A second class, associated with strong binding does not permit Au(I) reduction. The third class, presumably of intermediate binding strength, does permit gold reduction but only after elemental gold has accumulated elsewhere on the algal cell.

The effect of Au(0) accumulation on the binding ability of gold-bound algae was also investigated. In this experiment, algal cells were suspended at 2.0 mg/ml in 4.0 ml of 0.40 mM $HAuCl_4$. The samples were centrifuged, decanted and the gold-bound algae were then incubated for various lengths of time before being resuspended in a fresh solution of 0.40 mM $HAuCl_4$ solution. The results of this experiment are shown in Table I. It is apparent that the gold binding ability of gold-bound algae is enhanced by the accumulation of Au(0) on the algal cell. For example, if the gold-bound algae are resuspended in 0.40 mM $HAuCl_4$ without incubation, only 1.10 micromol of additional gold will be bound. In contrast, gold-bound algae that are incubated for 164 h before being resuspended, bind 1.34 additional micromoles of gold. This 22% enhancement of gold binding ability is not fully understood at present. One possible explanation is that gold may migrate from the binding site to a growing gold crystal during or after reduction, thereby freeing the binding site for additional gold binding. It is also possible, however, that gold atoms deposited on the algal cell during reduction of bound Au(I) serve as nucleation sites, and additional gold is deposited directly into a growing gold crystal without first binding to the algae. Either of these mechanisms would appear to be in agreement with the suggestion of Beveridge and Murray [21] who claimed that Au(III) bound on the cell walls of *Bacillus subtilis* initiates a seeding process which results in the formation of elemental gold. More work is needed in order to further clarify this mechanism.

TABLE I. The Effect of Au(0) Accumulation on the Binding Ability of Gold-bound Algae[a]

Incubation time[b] (h)	Supernatant [Au] (mol/l)	Additional Au bound (micromol)
0	1.26×10^{-4}	1.10
24	1.08×10^{-4}	1.17
48	0.98×10^{-4}	1.21
96	0.74×10^{-4}	1.30
164	0.66×10^{-4}	1.34

[a]Algae were suspended at 2.0 mg/ml in 4.0 ml of 0.40 mM $HAuCl_4$ (97% bound) then centrifuged, decanted, and incubated. Samples were then resuspended in 4.0 ml of 0.40 mM $HAuCl_4$ for 15 min, centrifuged, and the supernatant analyzed to determine additional Au bound. [b]Washed algae were also incubated to serve as gold-free controls. No significant change in binding was observed with time.

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