Nanoparticle factories: Biofilms hold the key to gold dispersion and nugget formation

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ABSTRACT

Biofilms living on gold (Au) grains play a key role in the biogeochemical cycle of Au by promoting the dispersion of Au via the formation of Au nanoparticles as well as the formation of secondary biomorphic Au. Gold grains from Queensland, Australia, are covered by a polymorphic, organic-inorganic layer that is up to 40 µm thick. It consists of a bacterial biofilm containing Au nanoparticles associated with extracellular polymeric substances as well as bacterioform Au. Focused ion beam (FIB) sectioning through the biofilm revealed that aggregates of nanoparticulate Au line open spaces beneath the active biofilm layer. These aggregates (bacterioform Au type 1) resulted from the reprecipitation of dissolved Au, and their internal growth structures provide direct evidence for coarsening of the Au grains. At the contact between the polymorphic layer and the primary Au, bacterioform Au type 2 is present. It consists of solid rounded forms into which crystal boundaries of underlying primary Au extend, and is the result of dealloying and Ag dissolution from the primary Au. This study demonstrates that (1) microbially driven dissolution, precipitation, and aggregation lead to the formation of bacterioform Au and contribute to the growth of Au grains under supergene conditions, and (2) the microbially driven mobilization of coarse Au into nanoparticles plays a key role in mediating the mobility of Au in surface environments, because the release of nanoparticulate Au upon biofilm disintegration greatly enhances environmental mobility compared to Au complexes only.

INTRODUCTION

Despite its low solubility in ground- and surface waters, Au is highly mobile in surface environments, leading to its enrichment in soils, calcrete, and placers (Reith et al., 2007; Mumm and Reith, 2007). Mineral colloids, nanoparticles, and bacterial cells may increase the environmental mobility of poorly soluble metals such as Cu or Au in surface environments (Weber et al., 2009; Honeyman, 1999). In addition, microbial biofilms create reactive geochemical boundaries of focused microbial activity and steep physicochemical gradients, where both increased and decreased rates of mineral weathering and metal mobilization occur (Little et al., 1997). We show for the first time that Au solubilization and formation of nanoparticulate Au is directly coupled within biofilms on Au grain surfaces. Biofilms act as effective nanoparticle factories, continuously recycling coarse Au and releasing nanoparticulate Au into the environment. This promotes Au dispersion and can explain the formation of geochemical anomalies observed in transported cover overlying deeply covered Au deposits (Reith et al., 2007).

Previous experimental studies have shown that bacterial biofilms increase Au mobility (Reith and McPhail, 2006; Fairbrother et al., 2009). Biofilms have been observed on Au pellets incubated in auriferous soils, where their presence led to a doubling of Au solubilization compared to unamended controls, yet mobilization and dispersion mechanisms remained unexplained (Reith and McPhail, 2006; Fairbrother et al., 2009). Cyano-, sulfide-oxidizing and sulfate-reducing, and metallophilic bacteria have been shown to biomineralize nanoparticulate Au from aqueous Au(I/III) complexes (Lengke and Southam, 2005, 2007; Lengke et al., 2006a, 2006b). In their presence, Au nanoparticles aggregate, transforming to Au crystals within weeks (Southam and Beveridge, 1994, 1996), and evolving over several months into coiled or wire Au with irregular and rounded structures that are morphologically similar to bacterioform Au (Lengke et al., 2007).

Gold grains or nuggets between 0.1 and 4 mm in diameter are the most abundant source of alluvial and eluvial Au and constitute economically important deposits such as the Witwatersrand deposit (Mossman et al., 1999). Lace-like, budding structures described as bacterioform Au occur on grains from many sites (Reith et al., 2007). Based on their morphology a microbial origin has been proposed (Fig. DR1A in the GSA Data Repository[1]) (Mossman et al., 1999; Watterson, 1992), which is consistent with evidence of Au addition during growth of secondary Au (McCready et al., 2003). However, Au nanocrystals and bacterioform morphologies have also been produced abiogenically, leading to the rejection of the biogeochemical aggregation model in favor of an abiogenic, detrital model (Watterson, 1994; Hough et al., 2007). In this model, Au grains and nuggets are hypogene in origin; their occurrence in surface environments reflects the weathering of rocks hosting primary mineralization, and their distribution depends upon mechanical accumulation in fluvial environments (Hough et al., 2007). They are dominantly Au/Ag alloys, but zones and coatings of high purity (up to 99.9 wt% Au) compared to the primary Au occur due to dealloying and mobilization of the Ag (Hough et al., 2007). Hence, additional evidence is required to understand the effect of microbial biofilms on Au mobility and biomineralization in order to put this 100-year debate to rest.

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[1]GSA Data Repository item 2010234, supplemental information (Figures DR1–DR4 and Movie DR1), is available online at www.geosociety.org/pubs/ft2010.htm, or on request from editing@geosociety.org or Documents Secretary, GSA, P.O. Box 9140, Boulder, CO 80301, USA.

MATERIAL AND METHODS

We obtained 100 Au grains, (mini-nuggets) from 0.2 to 1.5 mm in diameter (Fig. DR1A), from the Prophet gold mine in southeast Queensland, Australia (26°06′51.98″S, 152°17′0.94″E). The site is located in the North D'Aguilar Block of the New England orogen, and has a basement of late Paleozoic New England orogen subduction complex overlain by Permian sediments and intruded by Triassic dikes (Murray, 1987). The site was chosen for its detrital and secondary Au (Anderson, 1982). Gold grains were sampled according to a specific sampling procedure aimed at keeping biofilms intact and free from contamination. Each grain was washed five times on-site with sterile 0.9 wt% NaCl solution and stored in this solution. Samples were transported over ice to the laboratory, washed in sterile double-deionized water to remove salt, and air dried. Twenty grains were mounted on C tape and studied uncoated using focused ion beam–scanning electron microscopy (FIB-SEM; Helios NanoLab DualBeam, FEI, Netherlands, and LEO 1540 FIB-SEM, Zeiss, Germany). Sections were cut using a FIB at 30 keV and 21 nA, then surfaces were cleaned by decreasing the power of the beam to 30 keV and 2.8 nA, and finally 20 keV and 0.34 nA. Sectioned areas were element mapped by energy-dispersive X-ray spectroscopy (EDX) using a 10 mm^2 Sapphire Si(Li) EDAX detector. Electron backscatter diffraction (EBSD) analyses were conducted with a Zeiss Supra field emission (FE) scanning electron microscope (Zeiss, Germany) with an *e*-Flash detector, and data were analyzed using CrystAlign (Bruker AXS, Germany). For microprobe analyses, samples were embedded in epoxy resin and polished using 1 µm diamond paste and coated with a 15-nm-thick C film. The composition (Au, Ag, Hg, Fe) was quantitatively mapped using a Cameca SX50 microprobe (Cameca, France). Composition of microbial communities in biofilms was assessed using nested 16S rRNA polymerase chain reaction (PCR) denaturing gradient gel electrophoresis (DGGE) with targeted Sanger sequencing of DGGE bands and BLAST sequence analysis; sequences were deposited in the GenBank database (accession no. GU013673 to GU013680).

RESULTS AND DISUSSION

Gold grains obtained from the Prophet mine are typical for placer environments, displaying dark golden rims (10–100 µm thick) consisting of up to 99.9 wt% Au, and lighter-colored, Ag-rich cores containing up to 13.5 wt% Ag (Figs. DR1C and DR1D) (Southam et al., 2009). The grains also contain up to 2 wt% Hg with maximum concentrations in the core, reflecting the geochemistry of the primary mineralization in the region (Fig. DR1E) (Denmead, 1945). EBSD textural maps show that grain cores display polycrystalline structures with coherent and/or incoherent twin crystals (5–10 µm in diameter) and no internal compositional zoning (Fig. DR2A). Similar textures were observed in Au nuggets with masses of up to 8 kg and interpreted as indicative of high-temperature annealing (Hough et al., 2007). Hence, these textures provide strong evidence for a detrital origin of the Prophet mine grains.

The grains studied were collected under field-sterile conditions and treated to preserve the fragile biofilms. This procedure is in strong contrast to earlier studies that used acid (HF)–washed grains and nuggets from public or private collections. All 20 grains investigated with FIB-SEM were partially or entirely covered by an up to 40-µm-thick polymorphic layer (Figs. 1, 2, 3, and 4; Fig. DR3; Movie DR1). This layer consisted of sheet-like biofilms, Au nanoparticles and microcrystals, bacterioform Au, clay-, Fe-, and silicate minerals, and rare embedded transported sand grains (Figs. 2 and 3; Fig. DR3; Movie DR1). The biofilm community comprised up to 11 operational taxonomic units (OTUs) of which ten were identified as β-proteobacteria (Fig. DR4). The dominant organisms, present on >90% of DNA-positive grains, were *Delftia acidovorans* and *Cupriavidus metallidurans* (Fig. DR4). A recent study has shown that during its evolution, *D. acidovorans* coexisted with the heavy metal–resistant *C. metallidurans*, as there is evidence for horizontal transfer of genes involved in metal resistance and degradation of aromatic compounds between the species (van Houdt et al., 2009). Our results support these observations on an environmental level by demonstrating spatial coexistence of both species in a microenvironment for the first time. *C. metallidurans* also dominated bacterial communities on Au grains from other Australian sites in wet-tropical and temperate zones (Reith et al., 2006).

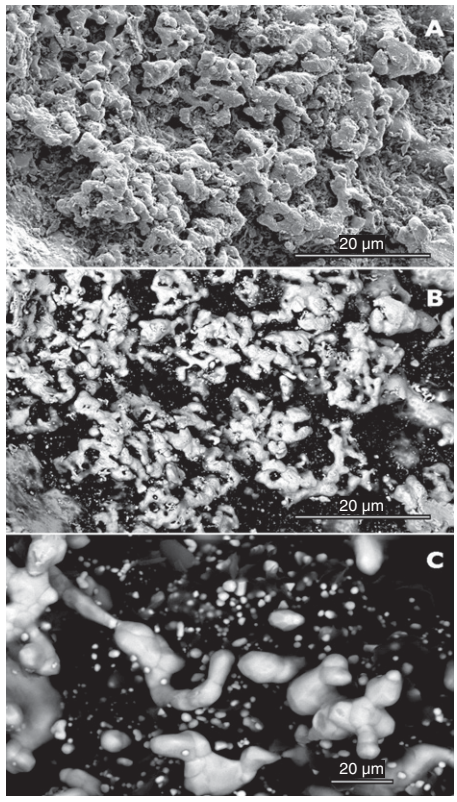

Figure 1. Secondary electron (A) and backscattered electron (B, C) micrographs of a polymorphic, organic-inorganic layer on a Au grain from the Prophet mine, Queensland, Australia, featuring nanoparticulate and bacterioform Au.

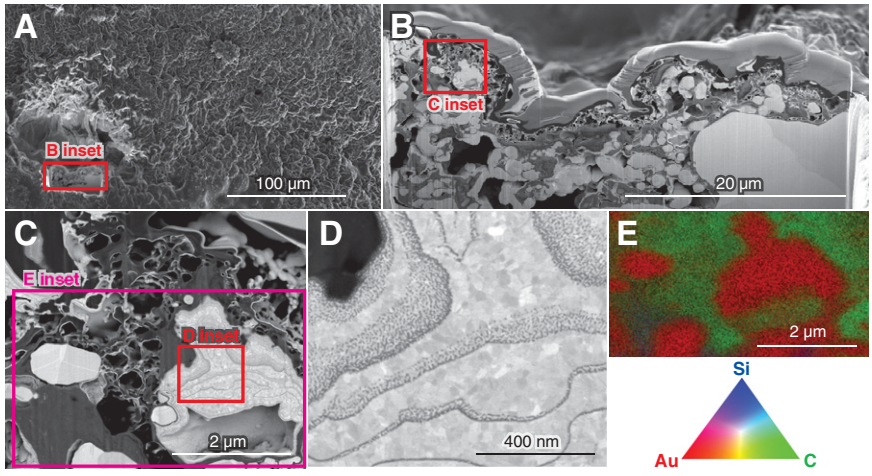

Figure 2. Secondary electron micrographs of a FIB-milled section of the polymorphic layer, showing a Au grain covered by a bacterial biofilm (A, B), aggregates of bacterioform Au(1) (C), bacterioform Au(1) displaying concentric crystallographic growth structures (D), nanocrystalline structure of this neoformed Au and element map of Au, C, and Si of a bacterioform aggregate Au(1) (E).

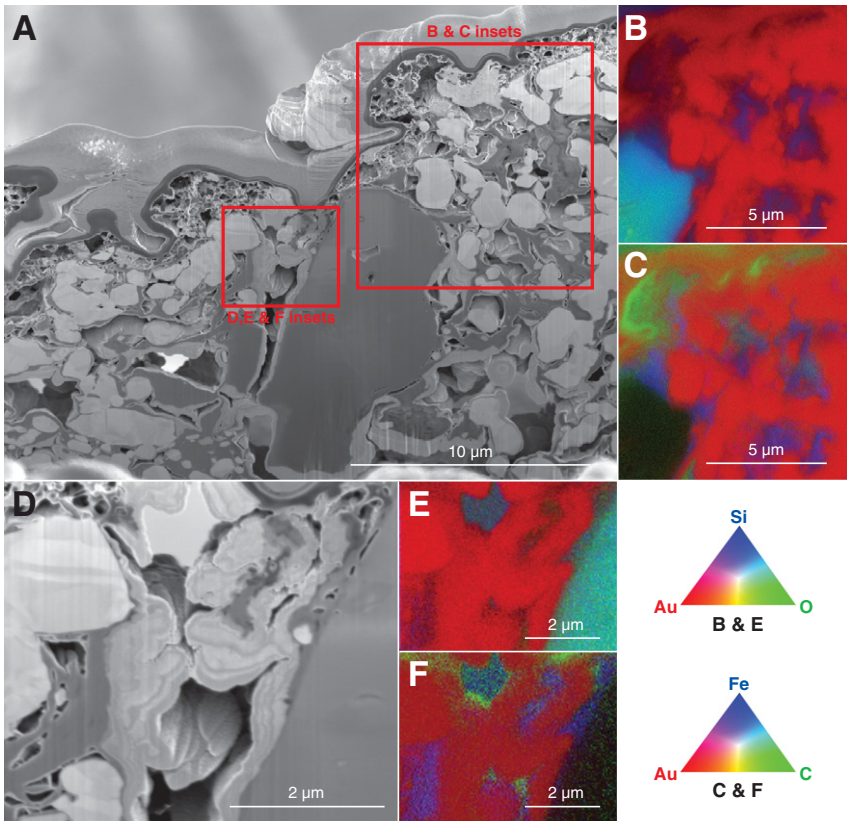

Figure 3. Secondary electron micrographs (A, D) and element maps (EDX; B, C, E, F) of a FIB-milled section showing a rounded sand grain that is enclosed by bacterioform Au(1).

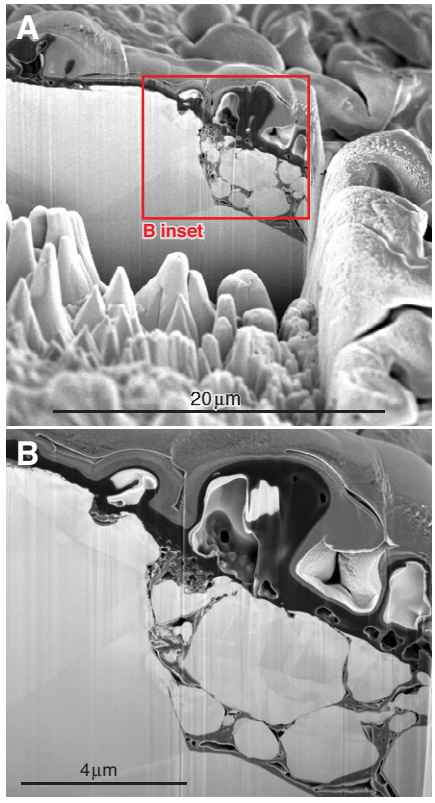

Figure 4. Secondary electron micrographs of a FIB-milled section of the polymorphic layer (A), showing bacterioform Au(2) formed by dissolution of underlying primary Au (B).

SEM imaging of the biofilm surface showed the presence of abundant nanoparticulate Au ranging from <10 to >200 nm in size that display a range of morphologies, including spherical particles, planar triangular Au nanoplates, and euhedral and octahedral microcrystals (Fig. 1C). Laboratory studies have shown how *C. metallidurans* takes up aqueous Au complexes, forming nanoparticles over a few days, likely as a result of active detoxification of Au complexes via efflux and reductive precipitation mediated by Au-specific operons (i.e., Rmet_4682–86 and Cop; Jian et al., 2009; Reith et al., 2009). The variety of morphologies for Au particles reflects the evolution observed over a few weeks in laboratory studies (Lengke et al., 2006b, 2007; Southam and Beveridge, 1994, 1996), and mediated by Au-binding proteins (Brown et al., 2000).

The Au locked in nanoparticles present on the surface of the biofilms may originate from dissolution of the underlying Au grain, but may also represent capture of soluble Au from solutions by metallophilic bacteria (McCready et al., 2003). To elucidate this we used FIB-SEM to obtain the first comprehensive insight into the internal structure and composition of the polymorphic layer. This method allows for the precise milling of sections across the fragile layer without disturbing internal structures; the section can then be imaged and chemically characterized. FIB-SEM revealed a complex history of Au solubilization, precipitation, and biomineralization within the layer (Figs. 2, 3, and 4; Fig. DR3; Movie DR1).

A zone of nanocrystalline Au aggregations was common beneath the active surface of the biofilm (Figs. 2B and 2C). These aggregations formed concentric growth structures (Figs. 2C and 2D) where an aggregate of nanocrystalline Au accumulated at the periphery of µm-scale voids. Element mapping showed that the aggregate was embedded in the carbonaceous matter of the biofilm, and that the void area also contained C (Fig. 2E). This suggests that a bacterial cell initiated the formation of nanoparticulate Au, resulting in cellular entombment by a layer of metallic Au, as shown in sections milled through a newly developing polymorphic layer (Movie DR1). We propose that the veneer of Au particles on the cell acted as nuclei for the attachment of mobile nanoparticles, leading to concentric growth of nanocrystalline Au aggregates and the formation of bacterioform Au(1). Figure 3 provides further evidence for the neoformation of bacterioform Au: A rounded, transported sand grain embedded in the biofilm was "overgrown" with bacterioform Au(1). EBSD mapping of unpolished Au grain surfaces covered by bacterioform Au(1 and 2) displayed randomly oriented Au nano- and microcrystals (Fig. DR2B). Distinctive Kikuchi patterns (EBSP, electron backscattered patterns) were obtained from individual crystals that displayed highly variable orientations compared to adjacent crystals; individual crystals analyzed were down to 35 nm in size (Fig. DR2B).

Direct evidence for the dissolution of primary Au was found at the contact between the primary Au and the polymorphic layer. Bacterioform Au(2) was in contact with the underlying primary Au, and the crystal boundaries observed in the primary Au were continuous into the solid buds (Fig. 4; Movie DR1). The buds consisted of coarsely crystalline Au, similar to the primary Au, suggesting that they formed via dealloying and dissolution of Ag/Hg from the primary Au alloy, and not via reprecipitation of dissolved Au. Toward the center of the polymorphic layer, buds consisting of high-purity Au containing C were observed (Fig. DR3). Although an origin via diagenetic transformation of nanoparticulate aggregates is possible, most of them appear to be "left over" from the dissolution of primary Au. In the latter case, the high Au contents resulted from Ag/Hg loss via dealloying, and the presence of C within the buds suggests that this process was biomediated.

CONCLUSIONS

This study reveals the importance of bacterial biofilms for catalyzing not only the formation and growth of secondary Au, but in particular the mobilization and dispersion of Au.

Regarding the formation of secondary Au grains, we conclude that the detrital and aggregation models need to be combined into a unified formation model for all alluvial and lateritic Au, hence resolving the debate concerning the formation of Au grains and nuggets. Primary Au grains and nuggets brought to the surface through weathering and/or mechanical transport are subject to biotic and abiotic processes leading to the solubilization of the primary Au and precipitation of secondary Au, thus forming the commonly observed grains/nuggets with a primary Au/Ag center and a secondary polymorphic layer. Biomineralization of mobile Au from solution/suspension enhances the growth of grains in surface environments, which may explain why secondary Au grains are often coarser than Au hosted in the associated primary mineralization. Weathering of primary sulfides can lead to the mobilization and dispersion of Au that was present as solid solution, to form pure secondary Au grains. The occurrence of bacterioform Au(2), which is the result of biomediated dealloying and Ag mobilization, may explain bud-like Au morphologies where no secondary Au occurs.

Most importantly, biofilms play a previously unrecognized role in promoting Au dispersion by acting as effective "nanoparticle factories". Since biofilms are subject to cycles of formation and destruction via desiccation or grazing by soil fauna, nanoparticulate Au will be periodically released and dispersed in the environment via transport in soil solutions and groundwater. This continuous release of nanoparticulate Au can explain the high mobility of Au in many near-surface environments, which leads to the formation of secondary Au deposits (McCready et al., 2003) and the development of geochemical halos around buried mineralization even in transported cover (Reith et al., 2007; Hough et al., 2008). This process can also serve as a natural analogue to understand the toxicology of manufactured Au nanoparticles released into the environment (Diegoli et al., 2008), and must be taken into account for estimating mobility of other rare, poorly soluble metals such as platinum group elements.

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