Stable silver isotope fractionation in the natural transformation process of silver nanoparticles

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Nanoparticles in the environment can form by natural processes or be released due to human activities. Owing to limited analytical methods, the behaviour of nanoparticles in the natural environment is poorly understood and until now they have only been described by the variations in the nanoparticle size or the concentration of the element of interest. Here we show that by using inductively coupled plasma mass spectrometry to measure silver (Ag) isotope ratios it is possible to understand the transformation processes of silver nanoparticles (AgNPs) in the environment. We found that the formation and dissolution of AgNPs under natural conditions caused significant variations in the ratio of natural Ag isotopes ([107]Ag and [109]Ag) with an isotopic enrichment factor (ε) up to 0.86‰. Furthermore, we show that engineered AgNPs have distinctly different isotope fractionation effects to their naturally formed counterparts. Further studies will be needed to understand whether isotope analysis can be used to reveal the sources of AgNPs in the environment.

AgNPs are the most widely used nanomaterial due to their broad spectrum in antimicrobial activities. An exponential increase in the environmental levels of AgNPs is predicted due to their rising usage in the ratio of natural Ag isotopes (107Ag and 109Ag) with approximately equal concentrations. To test this hypothesis, we studied two reversible processes of NPs. To test this hypothesis, we studied two reversible processes.

Here, we studied the variations in the stable Ag isotope ratio in the natural transformation process of AgNPs. Ag isotopes have previously been applied as chronometers for early-stage planetary differentiation and as tracers in archaeometry, whereas other applications are rare in the literature. Naturally occurring Ag is composed of 107Ag and 109Ag with approximately equal natural abundances (51.8% versus 48.2%) and subtle variations in the ratio of natural Ag isotopes ([107]Ag and [109]Ag) were observed in various terrestrial samples.

We first studied the Ag isotope fractionation caused by the formation of AgNPs from the reduction of Ag+ in the presence of DOM with Suwannee River humic acid (HA) as a model DOM and AgClO4 as a precursor. As shown in Fig. 2a and Supplementary Fig. 1, the exposure of the Ag+ solution to sunlight in the presence of HA quickly produced AgNPs, as evidenced by a wide absorption peak at ~440 nm corresponding to the feature surface plasmon resonance of AgNPs. The yield of AgNPs is strongly dependent on the HA concentration. Transmission electron microscopy (TEM) measurements indicated that the formed AgNPs were spherical particles with an average diameter of ~45 nm (Fig. 2b). The possible chemical reactions that account for the formation of AgNPs in natural waters are as follows:

\[
\text{HA}_{\text{red}} + \text{O}_2 \rightarrow \text{HA}_{\text{ox}} + \text{O}_2^- \quad (3)
\]

\[
\text{Ag}^+ + \text{O}_2^- \rightarrow \text{Ag}^0(\text{AgNP}) + \text{O}_2 \quad (4)
\]

where \(\text{HA}_{\text{red}}\) and \(\text{HA}_{\text{ox}}\) are the reduced and oxidized forms of HA as an example of DOM. Notably, we found that even at nanomolar concentrations of Ag+ the formation of AgNPs in natural waters was also possible (Supplementary Fig. 2).

Figure 2c shows the Ag isotopic compositions of AgClO4 and the formed AgNPs at different HA concentrations. The relative fraction of Ag+ that reacted was in the range of 5.9–20.6%. The δ109Ag value of AgClO4 was 0.15 ± 0.04‰ (mean ± 2 s.d., n = 3) and that of the AgNPs shifted positively, ranging from (0.72 ± 0.04‰) to (0.88 ± 0.04‰). This represented a significant fractionation (\(P < 10^{-5}\)) of Ag isotopes. The AgNPs were enriched in the heavy isotope and the residual Ag+ was enriched in the light isotope. Assuming that the fractionation followed the Rayleigh model, \(\varepsilon\) was estimated to be 0.63, 0.73 and 0.82‰ for HA concentrations of 5, 10 and 50 mg l\(^{-1}\), respectively (Supplementary Fig. 3).
As sunlight is an important factor in the formation of AgNPs in natural waters, the effects of sunlight were studied. We found that AgNPs could also form in the dark but the rate of formation was much slower (Supplementary Fig. 4). The formation of AgNPs in the dark also caused a significant Ag isotope fractionation ($P < 10^{-5}$; Fig. 2d) and the modelled $\varepsilon$ value in the dark was even

![Figure 1](image1.png)

**Figure 1 | Reactions and physical processes of AgNPs and Ag$^+$ in DOM-containing natural waters.** Ag$^+$ can be reduced to form AgNPs in a process mediated by DOM and sunlight, and the dissolution of AgNPs can release Ag$^+$. Meanwhile Ag$^+$ can also be transformed into AgO precipitates via photoreduction or adsorbed to the surface of AgNPs. Ag isotope fractionations caused by these processes were measured. The solid blue and dotted green arrows indicate chemical and physical processes, respectively.

![Figure 2](image2.png)

**Figure 2 | Ag isotope fractionation in the HA-mediated formation of AgNPs.** a, Absorption spectra of the AgClO$_4$ solution after exposure to sunlight for 48 h at varying concentrations of HA (0–50 mg l$^{-1}$). b, Typical TEM image of the formed AgNPs. Inset, a high-resolution TEM image of a single AgNP. c, Ag isotopic compositions of the formed AgNPs and the residual Ag$^+$ at different HA concentrations under sunlight. d, The effects of sunlight on Ag isotope fractionation in the formation of AgNPs using AgClO$_4$ (d) and AgNO$_3$ (e) as precursors. In c–e the percentages in parentheses represent the relative fraction of Ag$^+$ reacted (that is, the percentages of reduced Ag$^+$ in the total Ag). The bars start from the $\delta^{109}$Ag value of the initial Ag$^+$ concentration. The data in c–e are also given in Supplementary Tables 1 and 2 for a mass balance calculation. The error bars represent 1 s.d. from three parallel experiments ($n = 3$).
larger than that under sunlight (0.86‰ for the dark and 0.73‰ for sunlight; Supplementary Fig. 5). Given that counterions can affect the hydration structure and complex formation of Ag⁺ in solution²⁰, similar results were obtained (the modelled ε value was 0.48‰ for the dark and 0.32‰ for sunlight; Fig. 2e and Supplementary Fig. 5). Significant Ag⁺ isotope fractionation was also observed when lowering the initial concentration of Ag⁺ (Supplementary Fig. 6).

We then studied the dissolution of AgNPs in aqueous media. The experiment was carried out using both engineered polyvinylpyrrolidone-coated AgNPs (PVP-AgNPs; Supplementary Fig. 7) and HA-AgNPs (that is, AgNPs formed by the reduction of Ag⁺ with HA) as models of naturally formed AgNPs. The dissolution of AgNPs is a reverse reaction of the natural formation, in which dissolved oxygen plays a key role²¹:

\[
\text{Ag}^+ (\text{AgNP}) + \text{O}_2 \rightarrow \text{Ag}^+ + \text{O}_2^* \tag{5}
\]

Figure 3a,b show the kinetics of Ag⁺ release from PVP- and HA-AgNPs under sunlight and dark conditions. The mass of Ag⁺ released from both PVP- and HA-AgNPs reached a maximum in ~48 h, with PVP-AgNPs releasing more Ag⁺ than HA-AgNPs. The mass of released Ag⁺ showed a declining trend beyond 48 h. A purge of air into the solution did not change the declining trend (Supplementary Fig. 8), indicating that it was not resulting from the depletion of dissolved oxygen. This may be related to the readsorption of Ag⁺ to AgNPs. For PVP-AgNPs more Ag⁺ was released under sunlight than in the dark, probably because ultraviolet-irradiation-induced oxidation could degrade PVP to carboxylic acid and CO₂²². In contrast, HA-AgNPs released less Ag⁺ under sunlight than in the dark.

The Ag⁺ isotope fractionation in the dissolution of PVP-AgNPs is shown in Fig. 3c. In the dark no significant isotope fractionation was observed (P = 0.237). Under sunlight, the relative fraction reacted of AgNPs was close to that in the dark, but the fractionation was greatly aggravated with the released Ag⁺ being enriched in 109Ag (Δ109Ag = 0.66‰; P < 10⁻⁵). The initial concentration of AgNPs did not significantly affect the isotope fractionation (Supplementary Fig. 9). In the case of the HA-AgNPs (Fig. 3d), the fractionation was also minimal in the dark (Δ109Ag = −0.17‰; P = 0.014) but significantly larger under sunlight (Δ109Ag = −0.57‰; P = 10⁻⁷). This trend would be observed at different relative fractions of reacted AgNPs assuming an equilibrium fractionation model (Supplementary Fig. 10).

Notably, the released Ag⁺ from HA-AgNPs was enriched in 107Ag, which was opposite to that from PVP-AgNPs. In addition to PVP-AgNPs, we also studied the dissolution of citrate-coated AgNPs (Cit-AgNPs), another important type of engineered AgNPs. We found that the Ag⁺ released from Cit-AgNPs was enriched in 109Ag (Supplementary Fig. 11), which was consistent with PVP-AgNPs.

To figure out the role of Ag⁺ adsorption in the formation and dissolution of AgNPs we separately studied the Ag isotope fractionation caused by Ag⁺ adsorption in PVP and HA. We found that most of Ag⁺ in solution could be adsorbed to PVP or HA in ~2 h (Supplementary Fig. 12). As shown in Fig. 4a, adsorption of Ag⁺ to PVP or HA could result in a slight Ag⁺ isotope fractionation with the adsorbed Ag⁺ being enriched in 109Ag (Δ109Ag = 0.26‰ for HA and 0.20‰ for PVP; P < 0.01). Furthermore, the Ag⁺ isotope fractionation resulting from the photoreduction of Ag salts was also investigated to investigate the photosensitivity of Ag. Black Ag⁺ precipitation (non-nanosized without the feature surface plasmon resonance of AgNPs in absorption spectra) was observed when AgClO₄ and AgNO₃ solutions were exposed to sunlight. The photoreduction of Ag salts caused the remaining Ag⁺ to be enriched in 107Ag and the precipitates to be enriched in 109Ag (Δ109Ag = 0.20‰ for AgClO₄ and 0.27‰ for AgNO₃; Fig. 4b).

Possible mechanisms for the isotope fractionation caused by Ag⁺...
adSORption and the photoreduction of Ag salts are discussed in Supplementary Section 2.1. From the aforementioned results it is clear that the natural formation and dissolution of AgNPs are accompanied by significant Ag isotope fractionation. The observed fractionations could be comparable to the largest variations in the $^{109}$Ag/$^{107}$Ag ratio found in terrestrial samples—such as native Ag metal from globally distributed mines ($\sim$0.6‰)$^{12}$, Ag-fortified consumer products and environmental samples (up to 0.83‰)$^{16,23}$—and are much larger than those observed in the process of Ag$^+$ precipitation with Cl$^-$ (0.17‰)$^{24}$. Ag is highly reactive and may be involved in natural processes such as oxidation, sulfidation and precipitation. Taking into account the significant Ag isotope fractionation observed here and the increasing levels of AgNPs discharged into the environment$^{15,17}$, it is possible to infer that natural transformation processes of AgNPs may play an important role in natural Ag isotope fractionation.

Stable isotopic signatures could indicate the presence of concurrent processes. In Fig. 2d, the AgNPs formed in the dark were isotopically heavier than those formed under sunlight by 0.11‰ ($P = 0.039$), which could be attributed to the fractionation caused by the photoreduction of Ag salts (that is, the depletion of the heavy isotope in the solution; Fig. 4b). This could suggest that the formation of AgNPs under sunlight was accompanied by the photoreduction of Ag salts and that the apparent isotope fractionation was the joint outcome of these two processes (Fig. 1).

Stable isotope fractionation could provide insight into the mechanism of AgNP formation. In the natural formation of AgNPs the same fractionation direction (that is, the enrichment of the heavy isotope in the product) of the formation of HA-AgNPs as the adsorption of Ag$^+$ to HA could potentially explain why the HA-AgNPs were enriched in $^{109}$Ag and reveal the role of Ag$^+$ adsorption in the formation of HA-AgNPs (see Supplementary Section 2.2 for detailed discussion).

Stable isotope fractionation could also reveal the dissolution pathways of AgNPs. The dissolution of PVP-AgNPs under sunlight showed a significant enrichment of $^{109}$Ag in the released Ag$^+$ ($P < 10^{-2}$; Fig. 3c). This could be explained by a photo-oxidation-induced formation of an intermediate metal oxide phase (Ag$_2$O, for example) at the surface of the AgNPs$^{25,26}$. According to equilibrium isotope fractionation rules$^{19}$, the heavy isotope is prone to being concentrated in compounds with a higher oxidation state. As a result, the metal oxide phase should be enriched in $^{109}$Ag, thus causing the released Ag$^+$ to be enriched in $^{109}$Ag. However, the fractionation was not significant in the dark ($P = 0.237$; Fig. 3c). Such a difference between sunlight and dark conditions might suggest a different dissolution mechanism in the dark, such as a direct oxidation pathway in the absence of the formation of a metal oxide phase$^21$. Comparing the isotope fractionation effects in the dissolution of different AgNPs (Fig. 3c–d and Supplementary Fig. 11), the Ag$^+$ released from PVP- and citrate-AgNPs was enriched in $^{109}$Ag, whereas that from HA-AgNPs was enriched in $^{107}$Ag. This observation implied that there should be an extra process that depleted $^{109}$Ag in the released Ag$^+$ during the dissolution of HA-AgNPs. We noted that this effect matched the fractionation caused by the HA-mediated reduction of Ag$^+$, in which $^{109}$Ag$^+$ was preferentially reduced by HA (Fig. 2c–e). Therefore, we propose the dissolution pathways of AgNPs as in Fig. 5. For engineered AgNPs (Fig. 5a), their coatings (such as PVP and citrate), despite having some redox activities, are unable to reduce Ag$^+$.
under such mild natural conditions. Whereas for naturally formed HA-AgNPs (Fig. 5b), the HA coating can reduce Ag⁺ back to Ag₀ under natural conditions, in which ¹⁰⁹Ag is preferentially reduced over ¹⁰⁷Ag by the HA coating, thus leaving the final released Ag⁺ to be enriched in ¹⁰⁷Ag. These proposed pathways could also explain the results in Fig. 3a–b (see Supplementary Section 2.3).

From the discussion above, we learn that stable isotopes provide a unique tool for studying the processes of NPs. We inferred the underlying pathways from the apparent isotope fractionation effects. In contrast to methods that use isotopically enriched materials as tracers, this method employs natural variations of the stable isotope ratios and does not require any artificial tracers. However, it should be noted that extensive sample purification would be necessary before analysis of the Ag isotope ratios of samples from the natural environment.

Another important application of stable isotopes is source tracing. In this study, the observed δ¹⁰⁹Ag values of naturally formed and engineered AgNPs were in the ranges of 0.29–0.97‰ and 0.25–0.65‰, respectively, suggesting that δ¹⁰⁹Ag values could not directly indicate the exact sources of AgNPs. However, naturally formed AgNPs showed an inverse isotope fractionation direction compared with engineered AgNPs in dissolution (that is, the released Ag⁺ was enriched in different isotopes; Fig. 3). This result reveals the possibility of determining whether the AgNPs are naturally formed or engineered from their different isotope fractionation effects. Further studies are required to investigate whether or not high-precision Ag isotope analysis may be used as a technique to reveal the sources of AgNPs.

Methods

Methods and any associated references are available in the online version of the paper.

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Author contributions

Q.L. and G.J. conceived and designed the experiments; D.L. and T.Z. performed the experiments; Q.L. and D.L. analyzed the data; Y.C. and Y.Y. gave comments on the paper; Q.L. and G.J. wrote the paper.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to Q.L. and G.J.

Competing financial interests

The authors declare no competing financial interests.
Methods

Formation of AgNPs by the reduction of Ag⁺ with DOM. An aqueous borate buffer solution (0.2 M, pH 8.2, 200 ml) containing 200 mg l⁻¹ AgClO₄ and 10 mg l⁻¹ HA was prepared. For experiments in sunlight, the mixture was exposed to simulated sunlight in an Intelligent Illumination incubator (SAIFE Co., China) for 48 h. The irradiation intensity was 3000 LX, and the temperature was kept at 25 ± 1 °C. For experiments in the dark, the mixture was wrapped by aluminum foil and stored in dark for 7 days. The experiments were also carried out using AgNO₃ as a precursor. Three parallel experiments were performed (n = 3). All experiments in sunlight were carried out under both real and simulated sunlight and the same directions of Ag isotope fractionation were observed. As it was difficult to control the intensity of real sunlight, data were collected only under simulated sunlight.

Dissolution of AgNPs. The AgNPs used for dissolution experiments were first thoroughly rinsed with water using a centrifugal ultrafilter device (Amicon Ultra-15, Millipore, MA) until the concentration of Ag⁺ in the rinse solution did not change. Then, 9 ml of AgNP dispersion (500 mg l⁻¹) was sealed in a dialysis tube with a molecular weight cut-off (MWCO) of 7 kD and immersed in 900 ml of water with magnetic stirring to allow Ag⁺ release for up to 144 h. The Ag⁺ ions could pass freely through the dialysis tube but AgNPs could not. The dialysis process has been examined to ensure that no artificial Ag isotope fractionation was caused (Supplementary Table 6). The experiments were performed under both sunlight and dark conditions and three parallel experiments were performed (n = 3). The effect of purge with air or nitrogen on the dissolution of AgNPs was also studied.

Measurement of Ag isotope ratios by MC-ICP-MS. The Ag isotope ratio was measured using a Nu II Plasma MC-ICP-MS (Wrexham, UK) equipped with 16 Faraday cups and coupled to a DeSolvation Nebulizer System (DSN-100) working in a low-resolution mode. The optimized instrumental parameters are listed in Supplementary Table 7. All samples were diluted to an Ag concentration of 100 µg l⁻¹. Samples were introduced into the plasma by using a self-aspiration nebulizer at a flow rate of 0.11 min⁻¹. The procedure blank signals of Ag and Pd obtained in 3% (v/v) HNO₃ were subtracted from all sample and standard signals. Three parallel measurements were made for all samples (n = 3).

The mass bias was corrected based on a standard-sample-standard bracketing approach combined with the use of Pd as an internal standard, without assuming identical mass bias for Pd and Ag, as described previously. The ¹⁰⁹Pd/¹⁰⁹Pd ratio was used to correct the mass bias of ¹⁰⁹Ag/¹⁰⁷Ag ratio. As the true value of the ¹⁰⁸Pd/¹⁰⁶Pd ratio was unknown, the mass bias of ¹⁰⁹Pd/¹⁰⁶Pd ratio in two adjacent SRM 978a standard solutions was first corrected by the certified value of 1.07638 for ¹⁰⁷Ag/¹⁰⁹Ag in SRM 978a and their average value was then used for the correction of the ¹⁰⁹Ag/¹⁰⁷Ag ratio in the sample. The correction calculation was made by the Nu-Nice software (Wrexham, UK) based on the Russell exponential fractionation law:

\[
R_M = R_T \times \left( \frac{m_i}{m_j} \right)^{f(m)}
\]

where \( R_M \) and \( R_T \) are the measured and corrected ratios, respectively, \( m_i \) and \( m_j \) are the absolute masses of the isotopes of interest and \( f \) is the mass bias correction factor.

The Faraday cup configuration is given in Supplementary Table 8. Notably, ¹¹¹Cd was measured to mathematically remove possible interferences from ¹⁰⁶Cd and ¹⁰⁸Cd to ¹⁰⁶Pd and ¹⁰⁸Pd by using an iterative method (see Supplementary Section 1.7 for details). On the basis of this approach, a \( \delta^{¹⁰⁹}Ag \) value of 0.00 ± 0.04‰ (mean ± 2 s.d., n = 17) in a 100 ng g⁻¹ SRM 978a solution containing 100 ng g⁻¹ Pd was obtained. When the sample digestion process was included (Supplementary Table 6), the \( \delta^{¹⁰⁹}Ag \) value was still 0.00 ± 0.04‰ (mean ± 2 s.d., n = 5). The matrix effect in MC-ICP-MS measurement has also been evaluated (Supplementary Table 6). These results prove that this approach is highly accurate and precise for the measurement of Ag isotope ratios. It should be noted that if the method is applied to environmental samples, extra purification steps (for example, ion exchange columns) are recommended before MC-ICP-MS analysis due to the more complex sample matrix.