A Simple Entropic-Driving Separation Procedure of Low-Size Silver Clusters, Through Interaction with DNA

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Synthesis and purification of metal clusters without strong binding agents by wet chemical methods are very attractive for their potential applications in many research areas. However, especially challenging is the separation of uncharged clusters with only a few number of atoms, which renders the usual techniques very difficult to apply. Herein, we report the first efficient separation of Ag_2 and Ag_3 clusters using the different entropic driving forces when such clusters interact with DNA, into which Ag_3 selectively intercalates. After sequential dialysis of the samples and denaturalizing the DNA-Ag_3 complex, pure Ag_2 can be found in the dialysate after extensive dialysis. Free Ag_3 is recovered after DNA denaturation.

The emergence of new methods for the synthesis and isolation of noble metal atomic clusters has become promising for a wide range of biological, catalytic, energy and optical applications[1–8]. Additional advantages of these sub-nanostructures can be found in their small size and relatively low toxicity[9]. Which makes them very attractive in different fields, such as biological labels and bioimaging.[3–5] The characteristic size of a cluster is in the range of the electron de Broglie wavelength at the Fermi level (about 0.5 nm for Ag and Au). When this size is reached, the continuous density of states of the metal breaks up into discrete energy levels provoking a change from the conductive state to the characteristic semiconductor/molecular-like behavior.[10] This confinement of electrons in clusters results in discrete electronic transitions that provoke the appearance of quantum-size effects and molecular-like properties.

The effect of size on properties is especially significant for small clusters and explained by the large change of the HOMO-LUMO gap by increasing/decreasing only one atom in the clusters, especially in structures of less than ≈20 atoms.[10] The values of the gap, particularly in naked clusters, can be well approximated using the Jellium model.[10–12] Due to such expected dramatic dependence of properties with the number of atoms in the clusters, adequate and efficient procedures for their synthesis and purification must be developed to study and understand their physicochemical properties. Although water-soluble clusters without strong binding ligands and with a narrow size distribution have been successfully obtained,[13,14] preparation of water-soluble individual clusters without strong binding ligands (including synthesis and purification protocols), essential for the controlled study of their properties, is still a challenge.

We have previously shown that biological properties of small Ag clusters strongly depend on the number of atoms.[15,16] For example, Ag_3 clusters augment chromatin accessibility during DNA replication due to the fact that they intercalate reversibly to DNA. This effect was further used to increase the therapeutic index of chemotherapy in in vivo experiments.[17] In such studies, it was also shown that Ag_2 clusters, have only small interactions with the external groove of the helix, and do not display any obvious biological effect.

The aim of this work is to demonstrate the possibility of using the different binding properties to DNA of pre-formed small naked clusters, differing only in ONE atom, for their separation. We will show here that, due to the high specificity of this interaction, one can efficiently use it for the selective separation of uncharged Ag_2 and Ag_3 clusters in samples containing mainly Ag_2, Ag_3.

We firstly investigated the interaction of Ag_2 and Ag_3 with the GC and AT base pairs by DFT calculations. Figure S1 in the Supporting Information shows the central part of the DNA decamers, previously optimized[15,16] -see details in Supporting information-. It can be seen that the formation energy values are very similar for Ag_2 and Ag_3, so that enthalpy cannot be used as a driving force for a hypothetical separation of both

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Supporting information for this article is available on the WWW under https://doi.org/10.1002/open.202100028

type of clusters. However, we had observed before that the intercalation of Ag_3 into the base pairs of DNA extends its overall length considerably higher than other classical intercalators.[15] Therefore, such increase of unwinding and lengthening of the double helix will produce a subsequent increase in the water entropy due to the large entropic cost to order the hydrogen bonds around axially extended DNA. Therefore, the hypothetical separation of Ag_2 and Ag_3 will only be possible using such entropic driving force.

The proposed experimental method consists of sequentially dialyze a dispersion of the cluster-DNA adduct against water. By properly selecting experimental variables, such as time and temperature, different fractions could be separated and pure Ag_2 and Ag_3 fractions recovered confirming that the entropic driving force can indeed be used for the separation of both clusters. The efficiency of the separation was followed by Fluorescence Spectroscopy and Mass Spectrometry, which were chosen as characterization tools of the dialysis extraction products. For the experiments, an aqueous dispersion of low size silver clusters containing mainly two and three silver atoms was used, as previously reported.[15] The synthesis, based on kinetic control, was carried out by an electrochemical wet chemical method without capping ligands, and it is briefly explained in the Experimental Section of the Supporting information.

Figure 1a shows the UV/Vis spectrum of the cluster samples, in good accordance with those previously reported for a mixture of Ag_2 and Ag_3.[15,17] Figure 1b shows the fluorescence spectrum of the cluster samples. Two main bands, at 300 nm (4.13 eV) and 350 nm (3.54 eV), can be clearly distinguished. Using the Jellium model, the number of atoms, N, in the clusters can be calculated from the expression, $N=(E_F/E_g)^3$, where E_F and E_g are the Fermi level (5.4 eV for bulk Ag) and the HOMO-LUMO energy, which can be approximated by the emission peaks. From this formula and the estimated emission peaks, we can identify the presence of Ag_2 ($N=2.2$) and Ag_3 ($N=3.5$) clusters, respectively, as it was previously demonstrated.[15,17]

To further identify the presence of the clusters we carried out the ESI-TOF Mass Spectrometry analysis of the samples. Figures 1c and 1d show the peaks detected in the m/z ranges 350–500 and 520–670, respectively. After comparison with the blank of NaCl, introduced in the sample to facilitate the cluster ionization (see section 3a and Figure S2 in the Supporting Information), all the labelled peaks can be associated with Ag_2 (blue) and Ag_3 (red) clusters with different adducts (see Figures S3a–e and S4a–j in the Supporting Information for a detailed analysis of the peaks). Therefore, the mass spectra analysis confirms the presence of Ag_2 and Ag_3 clusters in the original samples.

The result obtained after the first dialysis (6 h in MilliQ water) is shown in Figure 2. It can be seen the presence of only the band at 300 nm in the emission spectrum, which indicates the presence of only Ag_2. This result shows the successful separation of Ag_2 from the initial cluster sample, as represented in the scheme of Figure 2. Subsequent dialysis shows no emission peaks, which evidence the absence of any cluster species, indicating the total separation of Ag_2 from the original sample just after the first dialysis. Finally, to separate Ag_3, it is necessary to thermally denature the DNA in order to separate the chains and liberate the intercalated Ag_3 clusters. For this, the DNA/Ag_3 mixture was heated at 96 °C for 15 min and immediately cooled to 0 °C to prevent renaturalization.

The last dialysis was performed in MiliQ water for 6 h at 0 °C. The results are shown in Figure 3. It can be now seen the presence of a main band at 350 nm, attributed to Ag_3, confirming the isolation of this cluster after the two previous dialysis.

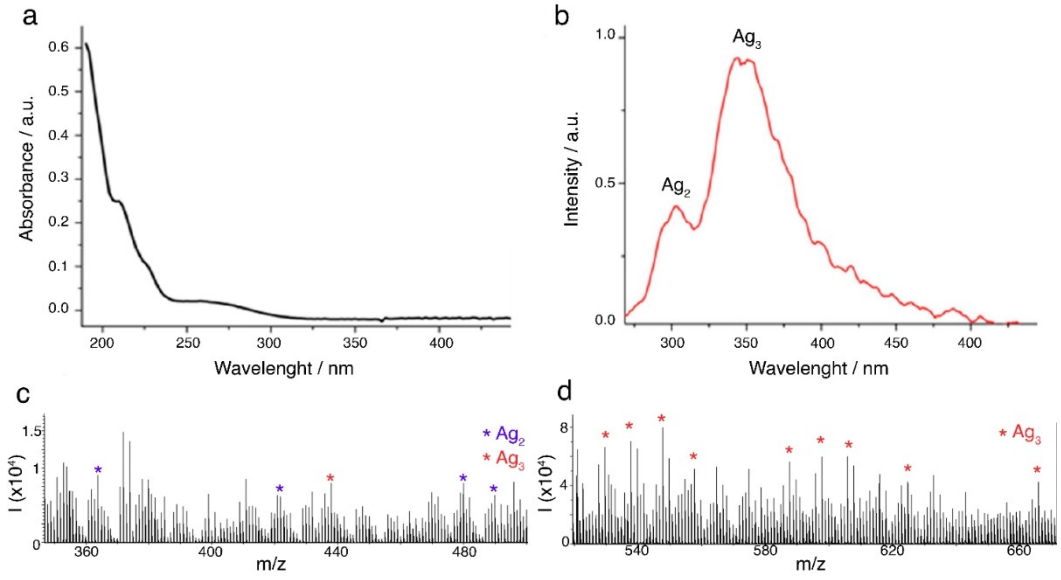

Figure 1. (a) Absorbance spectrum of the cluster sample and (b) fluorescence emission spectrum showing the characteristic emission peaks of Ag_2 and Ag_3. Mass spectra of the sample in the m/z ranges 340–500 (c) and 520–670 (d). Labelled peaks can be identified as Ag_3 (red) and Ag_2 (blue) clusters.

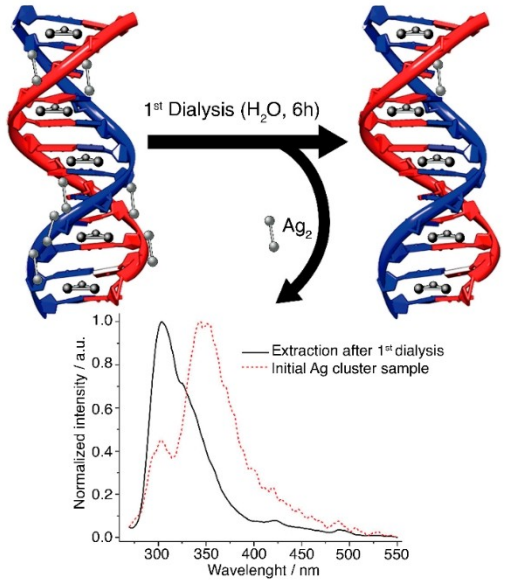

Figure 2. Scheme of the dialysis with DNA to separate Ag_2 clusters from a sample containing a mixture of both clusters (up). Emission spectra of the sample before and after dialysis (down).

Mass Spectrum analysis of this sample (Figure 3, see also section 3b in the Supporting Information for a detailed analysis of the peaks) shows very clearly the presence of only Ag_3 clusters (the much better resolution now is due to the purification of clusters after dialysis), confirming the separation of pure Ag_3 clusters from the initial sample.

To further confirm the presence of Ag_3 in the purified cluster samples we analyzed their effect on DNA mobility in an agarose gel. Due to the fact that Ag_3 intercalates into DNA, this affects its mobility in agarose gels.[16] As expected, DNA has less mobility in the presence of increasing Ag_3 cluster's concentrations (see section 4 in the Supporting Information). Moreover, Ag_3 reduced GreenSafe stain binding to DNA in agreement what we have found for doxorubicin (see Figure S7 in the Supporting Information), other DNA binding molecule.[16] Thus, confirming the presence of Ag_3 in the final purified cluster samples.

The results obtained confirm the hypothesis that separation of Ag_2 and Ag_3 is entropically driven due to the fact that Ag_3 clusters tend to intercalate between the DNA strands extending their length and increasing the entropy of the surrounding water. This explains the reason why Ag_2, lacking such entropy increase, elutes easier in the first dialysis. Finally, intercalated Ag_3 clusters could be extracted after denaturalization of DNA.

In summary, we can say that the present results demonstrate the importance of adding or subtracting just one atom from a cluster when dealing with subnanometric clusters. These different properties between Ag clusters with only one atom of difference allowed us to design a separation method, which would be very difficult to achieve with the common existing separation analytical tools.

Acknowledgements

We acknowledge the Consellería de Cultura, Educación e Ordenación Universitaria, Xunta de Galicia, Spain, Grupos Ref. Comp. (ED431C 2017/22); Agrupación Estratégica AEMAT (ED431E 2018/08); European Union's Horizon 2020 Research and Innovation Programme (Grant Agreement No. 825999); European Commission through FEDER and MICINN, Spain (MAT2017-89678-R). B.D. acknowledges a fellow from MICINN, Spain (BES-2016-076765). D.B. expresses gratitude for a postdoctoral grant from Xunta de Galicia, Spain (POS-A/2013/018).

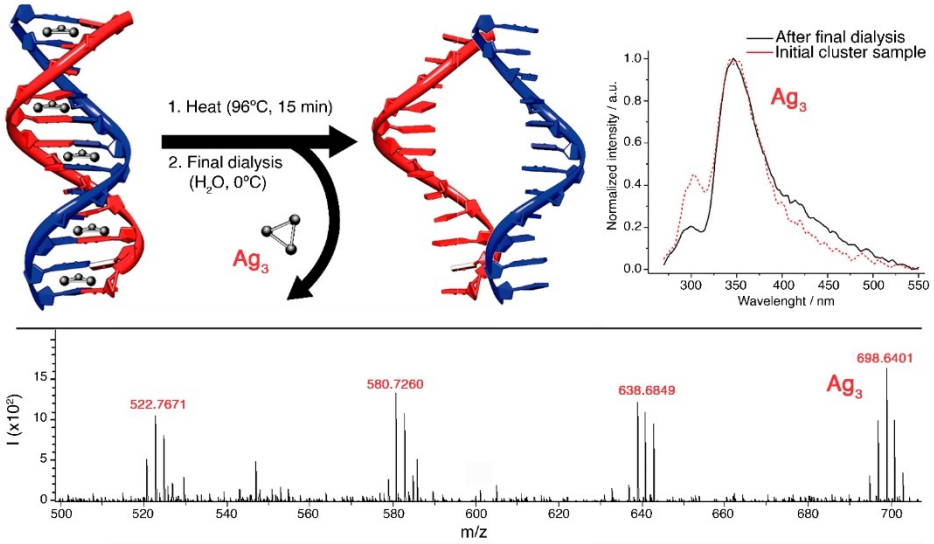

Figure 3. Scheme of the DNA denaturalization to recover Ag_3 clusters (up left). Emission spectra of the sample after the DNA denaturalization and subsequent dialysis (up right). Mass spectrum of the final sample showing only the presence of Ag_3 clusters (down).

Conflict of Interest

The authors declare no conflict of interest.

Keywords: DNA interaction · entropically-driven processes · mass spectrometry · silver clusters · separation procedures

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Manuscript received: February 2, 2021
Revised manuscript received: July 1, 2021