DNA-mediated biomineralization of a new planar Pt-complex

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The crystal growth morphology of a coordination complex of Pt(II) that crystallizes from solution can be controlled by using a second molecular species such as peptides or other organic compounds. Examples of crystal growth controlled by nucleic acids are few. In this article we describe the use of branched three-way junction (3WJ) DNA to influence the crystal growth of a planar platinum compound, *cis*-[(2, 2′-bipyridyl)*N,N*-di(2-hydroxyethyl)-*N′*-benzoyl-thioureatoplatinum(II)]chloride. Platinum complexes with extended planar aromatic residues are capable of stacking in the absence as well as in the presence of linear DNA double helices. This feature is based on the interaction of the compound with DNA through intercalation, resulting in the prevention of binding of DNA polymerase. Microscopic one-dimensional crystals were observed under these conditions. In the presence of the branched 3WJ DNA, however, additional nucleation sites are present, resulting in extended crystal growth of unique Pt compounds. At least two different crystal modifications were observed using transmission electron microscopy.

Introduction

We have recently reported on the binding of *cis*-[(2,2′-bipyridyl)*N,N*-di(2-hydroxyethyl)-*N′*-benzoyl-thioureatoplatinum(II)]chloride to DNA double helices.[1] We showed by DNA melting, circular dichroism, and DNA gel mobility studies, that in aqueous solution the Pt compound intercalates between successive Watson–Crick base pairs. The dilute aqueous solutions displayed some birefringence but no macroscopic crystals were formed. In the absence of DNA, however, transmission electron microscopy (TEM) revealed the formation of worm-like one-dimensional crystals (results not shown), the result of the association of the planar aromatic side groups through π–π electron interactions (stacking). In the presence of linear DNA double helices, the planar groups preferentially intercalate between the Watson–Crick base pairs similarly to the binding mode observed for planar organic dye molecules such as ethidium bromide. We expected the intercalated Pt-complexes to contribute strongly to the contrast of the images and so enhance the visualization of DNA double helices in the TEM. As we have a longstanding interest in the DNA three-way junction (3WJ) structures and now incorporate them into nanostructures, we sought to enhance the visualization of these DNA nanostructures through the intercalation of the Pt compound.

The TEM images observed in the absence and the presence of the branched DNA structures, respectively, showed two quite different crystal modifications. It seemed to us that the DNA helices at their branch-point could act as unique nucleation sites for inorganic Pt-complexes to form crystals that can become much larger than the initiating DNA junctions. This would be the first example of DNA-initiated biomineralization of a Pt-complex. Biomineralization is defined as the incorporation of inorganic crystals into an organic matrix.[2] The shape and growth pattern of the crystallized material is influenced and controlled by the presence of charged biological macromolecules.[3] The preferred choice of macromolecules associated with this process are proteins (the first RNA-mediated palladium-based structure was recently reported[4]). They act as nucleation centres for a particular kind of crystal growth that is directed by the initiation complex provided by the macromolecule.[5] Well-known biomineralization products are composites from inorganic compounds such as phosphates (for instance, apatite in teeth) or carbonates (that form mollusc shells) and proteins.[6–10] Progress in this field has recently been achieved through the use of macromolecules other than proteins and minerals (for example, magnetite[11]) to facilitate biomineralization. The control of crystal growth in these new materials is initiated by surfactants, lipid bi-layers, proteins, or polysaccharides.[3,9,12] Hitherto, no deoxynucleic acids have been implicated in biomineralization. However, macromolecular nucleic acids are well known to form large linear structures based on self-assembly of single strands through pairing of complementary bases.[13–16] The charge patterns created by uncommon nucleic acid assemblies (such as DNA three-way junctions[17,18]) provide nucleation sites for the formation of unique inorganic crystals of a Pt(II) coordination complex that would not form without the initiation sites.

Materials and methods

Nucleic acid synthesis

Oligonucleotide T1: 5′-CGCGGAGGAGGAAAGAAGAA-3′, oligonucleotide T2: 5′-CGCGTTCTTCTTCGGTCGGT-3′, and oligonucleotide T3: 5′-CGCGACCGACCGTCCTCCTC-3′ were synthesized using phosphoramidite chemistry with the help of a Beckman 1000M DNA synthesizer and the sequences were purified by DMT-on and DMT-off reverse-phase HPLC by means of an acetonitrile gradient. The extinction coefficients of these oligonucleotides were calculated using the nearest-neighbour model method, to be 256 900 M^{-1} cm^{-1}, 183 000 M^{-1} cm^{-1} and 184 300 M^{-1} cm^{-1}, respectively. These oligonucleotides were diluted to a final concentration of 100 μM with sterile MiliQ water and mixed in a 1:1:1 ratio to form DNA three-way junctions.

Three-way junction DNA ligation

Oligonucleotides T1, T2 and T3 were designed to contain matching sticky ends. The oligonucleotides were phosphorylated using Roche T4 polynucleotide kinase at 37°C overnight. The phosphorylated oligonucleotides were mixed in a 1:1:1 ratio. The stochiometric mixture was heated to 80°C for 1 min and cooled to 10°C at a cooling rate of 1°C per minute before ligation. The ligation was carried out by addition of Roche T4 DNA ligase. The process was allowed to proceed at 10°C for 30 min. The ligated DNA sample was purified by phenol extraction followed by ethanol precipitation and was consecutively dried. The dried samples were re-dissolved in sterile MiliQ water and solutions containing platinum compound, respectively. The samples were then observed by electron microscopy.

Platinum compound

The platinum compound *cis*-[(2,2′-bipyridyl)*N,N*-di(2-hydroxyethyl)-*N′*-benzoyl-thioureatoplatinum(II)]chloride was synthesized using the method described in *Wu et al.*[1,22] The compound contained a central platinum ion linked to two hydrophobic pyridine groups by dative bonds and a delocalized cyclic complex with hydrophilic sulphur–nitrogen groups (Fig. 1).

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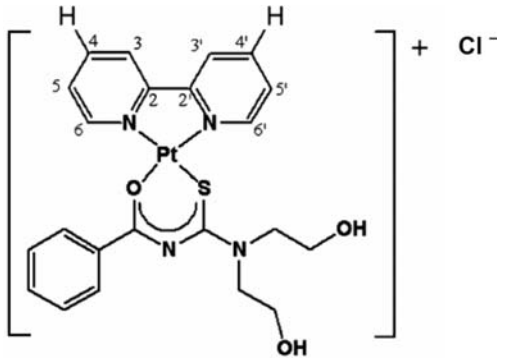

Fig. 1. Chemical structure of *cis*-[(2,2′-bipyridyl)*N*,*N*-di(2-hydroxyethyl)-*N*′-benzoyl-thioureatoplatinum(II)]$^{+}$ Cl^{-}.

Sample 1: the platinum compound and 3WJ DNA were mixed in a 1.5:1 ratio to final concentrations of 37.5 μM and 20 μM, respectively. The platinum complexes were pre-heated to 80°C and cooled before mixing with the DNA. Sample 2 contained the platinum compound only and was diluted to the same concentration as in Sample 1.

Electron microscopy

Sample 1 was then mixed with 0.2% benzyldimethylalkylammonium chloride and suspended on a carbon grid for 10 min. This sample was washed by dipping the grid into 90% ethanol for 15 s and airdried. Sample 2 was suspended on a carbon grid and air dried. The dried samples were then examined by bright field transmission electron microscopy.

Results

Figure 2a shows crystals of the platinum compound grown in the absence of nucleic acids. The platinum complexes are positively charged and formed self-stacked arrangements through hydrophobic interaction between the heterocyclic rings (Fig. 1). These compounds aggregated into a family of similar crystal structures with irregular polygonal shapes (Fig. 2b). The individual crystals adopted a pyramid-like shape with planar faces (Fig. 2c) and assembled in a dendrimer-like pattern, but they were not connected. Their size and shape indicates that they resulted from an initial crystal formation followed by rapid growth in three dimensions into rod-like structures that differed in size. The polygonal crystals further aggregated (Fig. 2a) into large complexes.

In the presence of nucleic acids, however, the crystals of the platinum complexes intercalated into the DNA adopted a quite different morphology (Fig. 3a). The shape of the aromatic side groups allowed them to insert themselves between the base pairs of the double-stranded DNA; this binding mode is similar to the intercalation mechanism observed for ethidium bromide.[19] The positive charges of the cationic platinum compounds stabilize the complex through ionic interactions with the negatively charged phosphate backbone of the DNA. The high charge density and the special geometry of the charge arrangement at the branch point of the DNA structure acts as a unique organizer of the initiation complex that can grow into a crystal modification that is unobtainable in the absence of this organizer. It is this unique nucleation process that leads to completely new structural arrangements (Fig. 3a, b).

Conclusion

Biological macromolecules, by their special properties as polyelectrolytes, play an important role in biomineralization processes as they provide a regular charge pattern localized

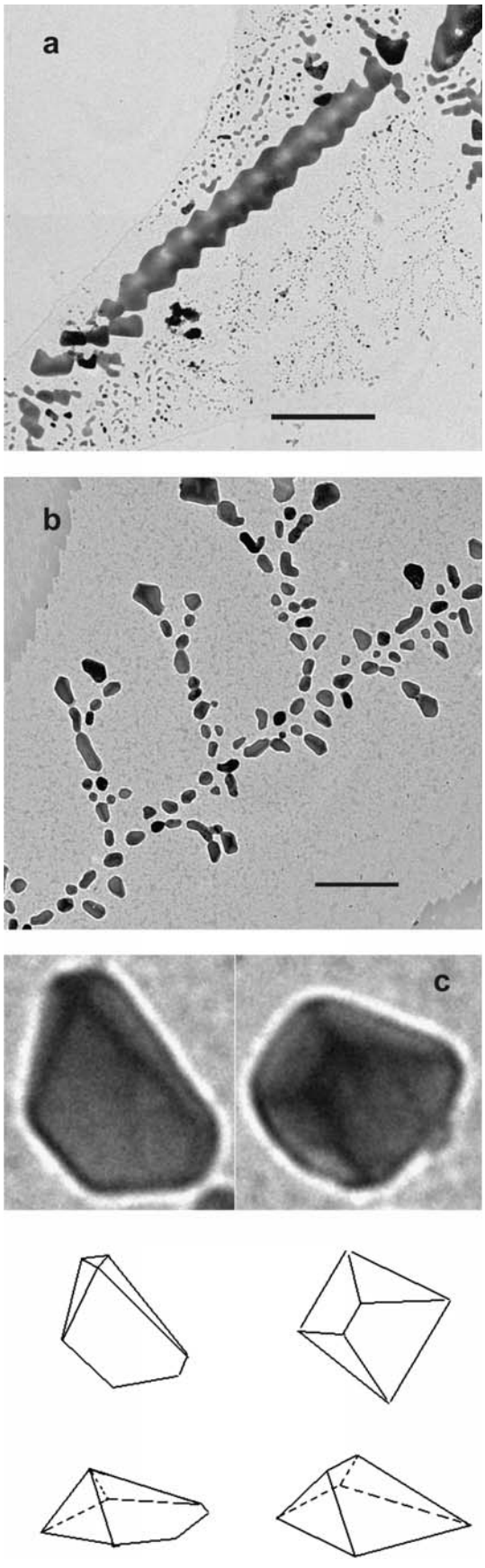

Fig. 2. **a**, Electron micrograph of a large crystal aggregate of the platinum compound formed in the absence of a DNA three-way junction. Scale bar, 2500 nm. **b**, Ensemble of pyramidal platinum compound crystals formed on a carbon grid from an air-dried platinum solution. Scale bar, 1000 nm. **c**, Selected pyramidal crystals as observed in an electron micrograph, and corresponding line drawings to accentuate their geometry.

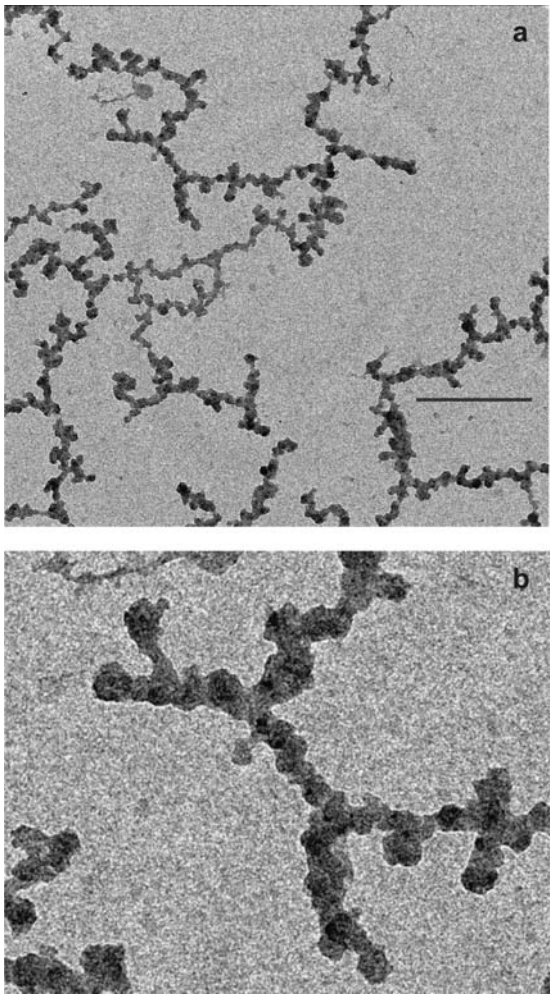

Fig. 3. **a**, Electron micrograph of a sample of platinum compounds nucleated by DNA three-way junction networks. Scale bar, 250 nm. **b**, Enlarged image of the platinum compounds in (a).

along the macromolecule's surface that is missing in normal crystallization in solution. In the experiment discussed in this paper, the platinum compounds in the absence of branched DNA crystallize according to the Ostwald step rule.[20,21] This rule applies to a growth mechanism that is based on the spontaneous formation of a seed crystal (initial state), followed by crystal growth in the direction of the pre-formed crystal faces (growth state). In the presence of the DNA three-way junction (and in general in the presence of charged biological macromolecules with a localized surface-charge pattern such as proteins or nucleic acids), the mechanism of crystal formation and growth is different. The seed is provided by the charged linear polymer. Growth results from metal ions diffusing onto the polymer lattice. Recently, Gugliotti *et al.*[4] described an experiment that demonstrated the special crystal formation of Pd compounds guided by the surface charge distribution of RNA aptamers. The pre-formed charge pattern initiates a new crystal modification of the palladium compound that cannot form spontaneously in solution. It can arise only because of the pre-formed cluster of charges on the surface of the aptamer. The results reported in this paper for the Pt compounds interacting with a branched DNA helix are comparable to the results obtained by Gugliotti's group, and can be understood as resulting from a similar mechanism of macromolecule-guided crystal formation. It will be interesting to see whether this newly observed example of biomineralization in the test tube can be found in nature as well.

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