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Nanoparticles of Prussian Blue Ferritin: A New Route for Obtaining Nanomaterials

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The dissociation of apoferritin into subunits at pH 2 followed by its re-formation at pH 8.5 in the presence of hexacyanoferrate(III) gave rise to a solution containing hexacyanoferrate(III) trapped within the apoferritin and hexacyanoferrate(III) outside it. The addition of Fe^{II} to the dialyzed solution resulted in the appearance of the characteristic Prussian blue color. The UV–vis spectrum of this solution showed a broad band centered at 710 nm, and the IR spectrum contained a broad–medium band at 2083 cm^{-1}. Both features are consistent with the charge-transfer band and the C–N stretching mode in the Fe^{II}–CN–Fe^{III} fragment of PB. TEM images of the obtained Prussian blue solution showed discrete spherical electron dense iron particles with an average size of about 5 nm. This represents a new route for preparing metallic nanoparticles that offers control over the size and protection against aggregation. Moreover, the fact that the particles are obtained by reaction of hexacyanoferrate(III) and iron(II) building blocks opens up the possibility of obtaining not only homo- but also heterobimetallic nanoparticles.

Nanoscale metal particles are attracting considerable attention for their intriguing properties and potential applications. These nanoparticle materials often exhibit very interesting magnetic, electrical, optical, and chemical properties that cannot be achieved by their bulk counterparts.[1] The size and properties of the nanoparticles confer them potential technological applications in a wide range of fields, including magnetic memory devices[2] and magnetic resonance imaging.[3]

From a synthetic point of view, the main challenge is to seek new procedures that allow the preparation of nanoparticles in a controlled manner, obtaining (i) a narrow size distribution, because the properties of nanoparticles are highly size dependent, (ii) protection against aggregation, and (iii) a wide range of chemical compositions.

One possible route to the preparation of an ideal isolated nanomaterial with homogeneous size is the use of a preorganized biomolecular matrix as a chemically and spatially confined environment for its construction. Good examples of this strategy have been reported by Mann, Douglas, et al., who have developed synthetic routes to produce nanoparticles within apoferritin. Apoferritin consists of a spherical protein shell composed of 24 subunits surrounding an aqueous cavity with a diameter of about 8 nm capable of accommodating around 4500 iron atoms, giving rise to ferritin, the major intracellular storage form of iron.[4] Channels are generated by the multisubunit construction of the apoferritin shell. Eight hydrophilic channels of about 4 Å lead to the protein cavity. Mann, Douglas, et al. have mainly adopted two different approaches to produce nanoparticles within the protein cage of apoferritin: (i) in situ transformation of the hydrated iron(III) oxide core of native ferritin by reaction with an appropriate reactant capable of penetrating into the internal cavity and (ii) redox-driven reactions involving metal-ion uptake and deposition into the apoferritin cavity. By the first approach, iron sulfide particles are generated within the ferritin,[5] whereas the second route allows the preparation of biomimetic ferritins reconstituted with non-native inorganic compounds such as oxyhydroxides of iron,[6] manganese,[7] uranium,[8] and cobalt.[9]

We describe here a new chemical method for the preparation of metal nanoparticles also based on the apoferritin cage but using a rather different approach (Scheme 1). The apoferritin protein is loaded with a metal complex and isolated. This loaded apoferritin can act as a reactor because the trapped metal complex is able to react with a second

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(1) Ashoori, R. C. *Nature* **1996**, *379*, 413 and references therein.
(2) Speliotis, D. E. *J. Magn. Mater.* **1999**, *193*, 29.
(3) Portet, D.; Denizot, B.; Rump, E.; Lejeune, J. J.; Jallot, P. *J. Colloid Interface Sci.* **2001**, *238*, 37.
(4) Harrison, P. M.; Andrews, S. C.; Artymuik, P. J.; Ford, G. C.; Guest, J. R.; Hirzmann, J.; Lawson, D. M.; Livingstone, J. C.; Smith, J. M. A.; Treffery, A.; Yewdall, S. J. *Adv. Inorg. Chem.* **1991**, *36*, 449−486.
(5) Douglas, T.; Dickson, D. P. E.; Betteridge, S.; Charnock, J.; Garner, C. D.; Mann, S. *Science* **1995**, *269*, 54−57.
(6) Meldrum, F. C.; Heywood, B. R.; Mann, S. *Science* **1992**, *257*, 522−523.
(7) Mackle, P.; Charnock, J. M.; Garner, C. D.; Meldrum, F. C.; Mann, S. *J. Am. Chem. Soc.* **1993**, *115*, 8471−8472.
(8) Meldrum, F. C.; Wade, V. J.; Nimmo, D. L.; Heywood, B. R.; Mann, S. *Nature* **1991**, *349*, 684−687.
(9) Douglas, T.; Stark, V. T. *Inorg. Chem.* **2000**, *39*, 1828−1830.

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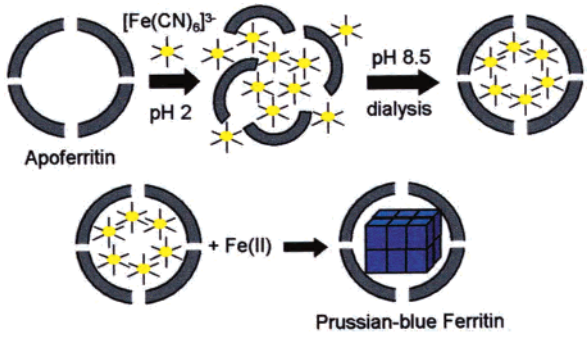
Scheme 1. Schematic Representation of the pH-Induced Dissociation−Re-formation Process of Apoferritin in the Presence of Hexacyanoferrate(III) and the Subsequent Reaction with Iron(II) Giving Rise to Prussian Blue Complex within the Apoferritin Cavity



metal ion to build a new material. This method has a clear advantage over those reported by Mann et al.,[5−9] also based on the apoferritin cage: the great versatility in the chemical composition of the nanoparticles. This is so because the formation of the nanoparticle takes place through the reaction of two metal complex building blocks.

We chose hexacyanoferrate(III) as the metal complex to be trapped by the apoferritin and iron(II) as the second metal ion. This choice was based on the well-known fact that reaction between hexacyanoferrate(III) and iron(II) leads to the Prussian blue complex (PB), which can be easily detected by UV−vis and IR spectroscopy because of its characteristic color and C−N stretching bands. In addition, PB is the paradigm of a recurrent complex in coordination chemistry mainly due to its large number of interesting properties and applications. Nowadays, PB derivatives play a crucial role in the field of high-T_c molecule-based magnets.[10]

A route for trapping molecules within apoferritin has been previously reported,[11,12] consisting of the dissociation at pH 2.0 of the apoferritin into its 24 subunits in the presence of the molecule to be trapped, followed by its reconstruction at pH 7.0−9.0. We followed the same scheme to load the apoferritin with hexacyanoferrate(III) (Scheme 1).[13]

The starting apoferritin and hexacyanoferric(III) acid concentrations were 5.66×10^{-5} and 0.1 M, respectively. Once the apoferritin was reconstructed in the presence of hexacyanoferrate(III), two types of hexacyanoferrate(III) could be detected: one trapped by the apoferritin and the other outside the apoferritin, in the external solution. The resulting yellow solution was exhaustively dialyzed against four changes of water.

During this treatment, the yellow color within the dialysis bag rapidly decreased until it remained constant with subsequent dialysis, indicating that hexacyanoferrate(III) readily diffused into the external solution. In fact, the addition of an iron(II) salt to this external solution gave rise to the characteristic Prussian blue color. However, even after extensive dialysis, the easily discernible yellow color of hexacyanoferrate(III) remained associated with apoferritin in the dialysis bag, indicating the existence of hexacyanoferrate(III) trapped by the apoferritin. The iron concentration, measured by atomic absorption, was 2.0 mM, and the apoferritin concentration, determined by the Lowry total protein micromethod (Sigma diagnostic), was 2.3×10^{-5} M. It can be concluded, therefore, that about 90 hexacyanoferrate(III) anions were trapped per apoferritin. This value is much higher than the theoretical one proposed by Watt et al.,[11] of 8.7 molecules per apoferritin, which was calculated by considering the size of the apoferritin cavity and the concentration of the molecule to be trapped and ignoring the latter's size or interaction with the apoferritin subunit. This result suggests the existence of a specific interaction between the subunits and hexacyanoferrate(III) in the apoferritin reassembly process. This interaction must occur at the internal surface of the subunits rather than at the external surface because prolonged incubation of apoferritin (2.3×10^{-5} M) with potassium hexacyanoferrate(III) (0.1 M) at pH 7.0 followed by exhaustive dialysis (as in the loading experiment) led to a colorless solution that did not produce the Prussian blue color after addition of iron(II).

Following Scheme 1, an iron(II) perchlorate hydrate solution (3.0 mM) was slowly added to the dialyzed solution containing the hexacyanoferrate(III)-loaded apoferritin,[13] producing the immediate appearance of an intense blue color. The resulting solution was dialyzed against two changes of water. The UV−vis spectrum showed a broad band centered at 710 nm, and the IR spectrum of the lyophilized solution contained a broad−medium band at 2083 cm^{-1}. Both features are consistent with the charge-transfer band and the C−N stretching mode in the Fe^{II}−CN−Fe^{III} fragment of PB.[14]

Transmission electron microscopy (TEM) images (Figure 1) of samples taken from the blue solution show discrete spherical electron dense particles of relatively homogeneous size (Figure 2). The particles are light-sensitive, thus preventing the attainment of a good electron-diffraction pattern. Energy-dispersive X-ray analysis (EDXA) showed that the material contained iron (Figure 2). The spherical form, size, and composition of the nonaggregate particles validated our procedure to make nanoparticles inside the apoferritin: the reaction between hexacyanoferrate(III) and iron(II) did not

(10) Mallah, T.; Thiebaut, S.; Verdaguer, M.; Veillet, P. *Science* **1993**, *262*, 1554−1557. Verdaguer, M.; Bleutzen, A.; Marvaud, V.; Vaissermann, J.; Seuleiman, M.; Desplanches, C.; Scullier, A.; Train, C.; Garde, R.; Gelly, G.; Lomenech, C.; Rosenman, I.; Veillet, P.; Cartier, C.; Villain, F. *Coord. Chem. Rev.* **1999**, *190−192*, 1023.

(11) Webb, B.; Frame, J.; Zhao, Z.; Lee, M. L.; Watt, G. D. *Arch. Biochem. Biophys.* **1994**, *309*, 178−183.

(12) Aime, S.; Frullano, L.; Crich, S. G. *Angew. Chem., Int. Ed.* **2002**, *41*, 1017−1019.

(13) Horse spleen apoferritin was purchased from Sigma. An apoferritin solution (3 mL, 5.66×10^{-5} M), was gradually adjusted to pH 2 by slow addition of HCl solution (0.1 M). This pH was maintained for 20 min. At this point, hexacyanoferric acid (22 mg) was very slowly added and then the pH of the mixture was slowly raised to 8.5 by addition of a NaOH solution 0.1 M. The resulting solution was stirred at room temperature for 2 h and then exhaustively dialyzed for 4 days against several changes of water using a Spectra/Por Float-A-Lyzer with a molecular weight cutoff (MWCO) of 25000 Da. After this time, 0.9 mL of the solution was again dialyzed against 0.9 mL of water. After 12 h, the dialysis reservoir solution was collected and mixed with a freshly prepared iron(II) perchlorate hydrate solution (0.1 mL, 0.03 M). The resulting solution did not show the characteristic Prussian blue color, and, therefore, the concentration of the nontrapped hexacyanoferrate(III) was considered negligible. A Philips CM-20 HR analytical electron microscope operating at 200 keV was used.

(14) Rosseinsky, D. R.; Lim, H.; Zhang, Z.; Jiang, H.; Chai, J. W. *Chem. Commun.* **2002**, 2988−2989.

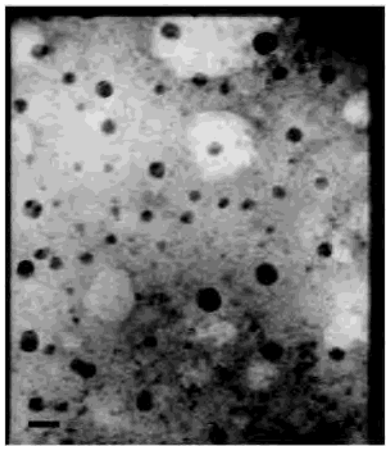


Figure 1. TEM image of Prussian blue nanoparticles (scale bar 10 nm).

lead to an infinite 3D well-ordered PB crystal structure but to a confined "ferritin PB complex". Discrete rounded cubic particles of larger size were also observed (average diameter 60 nm). EDXA confirmed that these particles also contained iron. The reason that the size of these cubic rounded particles was so large remains unclear at the moment. At any rate, it is interesting to note that these particles were in marked contrast to those obtained in a control experiment: in the absence of apoferritin, the synthesis of PB complex gives a blue bulk precipitate that consists of cubic particles >100 nm in diameter with a broad size distribution.

In conclusion, we present a new chemical route for preparing PB nanoparticles protected against aggregation. This strategy could be extrapolated to obtain other homo- and heterobimetallic nanometer systems. It should be noted that, only recently, Mann et al. have succeeded in preparing heterobimetallic nanoparticles of PB derivatives via a different method that does not make use of a biological preorganized matrix.[15] The Mann method leads to cubic nanoparticles whereas in our method the presence of apoferritin induces the formation of spherical bionanoparticles.

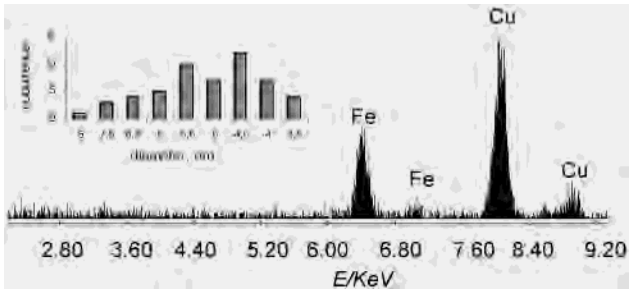


Figure 2. Particle size distribution for PB nanoparticles formed inside the apoferritin cavity and EDXA spectrum of the nanoparticles showing the presence of iron corresponding to PB. Copper peaks are due to the sample grid.

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(15) Vaucher, S.; Li, M.; Mann, S. *Angew. Chem., Int. Ed.* **2000**, *39*, 1793−1796. Vaucher, S.; Fielden, J.; Li, M.; Dujardin, E.; Mann, S. *Nano Lett.* **2002**, *2*, 225−229.