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Colloids, helices, and patterned films made from heme proteins and manganese oxide

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New colloidal materials with enzyme-like peroxidase activity were made from octahedral layered manganese oxide and myoglobin and hemoglobin, and were converted to macroscopic helices and patterned films.

Self-assembly is a demonstrated strategy for making meso- and macroscopic structures from nanometer sized building blocks.[1] Nanoscale composites of inorganic materials and functional biomolecules might provide structurally unique, stable nanostructured materials with applications in biocatalysis, biosensors, and electronics. Herein we report that octahedral layered (OL) manganese oxide nanoparticles intercalated by tetraalkylammonium (TAA) cations formed new colloidal materials with the proteins myoglobin (Mb) and hemoglobin (Hb). Intercalated protein–manganese oxide colloids retained the peroxidase activity of the native proteins. Under appropriate conditions, the colloids were converted to macroscopic helical structures and patterned films (Figs. 1 and 2).

In 1999, Suib *et al.* first described layered manganese oxide nanomaterials intercalated by tetraalkylammonium ions (denoted as TAAOL-1) made from the reduction of tetraalkylammonium permanganate salts dispersed in water–alcohol mixtures.[2,3] Transmission electron microscopy and small angle neutron scattering revealed disk-shaped particles with diameters of 20–120 Å, controlled by the identity of intercalant ion and synthetic conditions. If tetramethylammonium ion was used, these dispersions could be converted to helices and rings when heated in glass capillary tubes.[4,5] These unusual macroscopic structures did not form when TAAOL-1 was prepared with any other type of tetraalkylammonium ion.

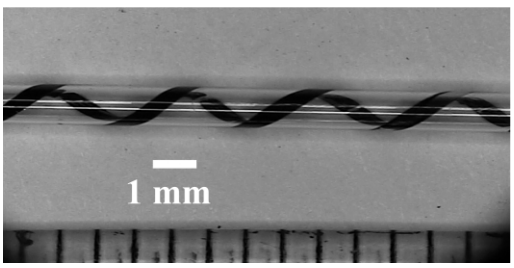

Fig. 1 Helix formed by heating Hb-TMAOL-1 composite in capillary at 85 °C for one week (ruler on bottom).

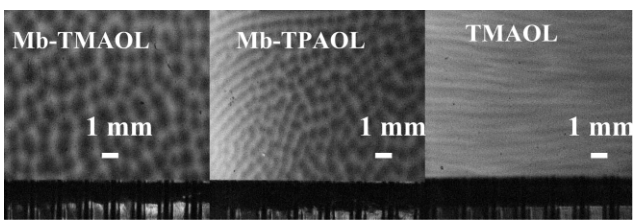

Fig. 2 Optical micrographs of films made by casting dispersions on glass slides and drying.

Enzymes are efficient, specific catalysts whose practical use is facilitated by attachment onto solids.[6] Interactions of enzymes with inorganic nanoparticles could result in important new nanostructured materials on nano-, meso- and macroscopic size scales with unique structures and activities. For example, protein–lipid complexes[7] and proteins adsorbed into α-zirconium phosphate galleries[8] showed enhanced catalytic activities. TAAOL-1 materials are good candidates for components of bioinorganic materials because of their layered structures into which enzymes might be inserted, high surface areas, and simple preparation methods. Preliminary work showed a very high affinity of TAAOL-1 nanoparticles for positively charged proteins such as myoglobin and hemoglobin . While Mb (one iron heme per molecule) and Hb (4 iron hemes per molecule) play major roles in mammalian oxygen transport and storage, they also catalyze enzyme-like oxidations of organic molecules.[9]

Dispersions of TAAOL-1 nanoparticles[2,3] (5.0 mL, 0.1 M in Mn, TAA alkyl groups = methyl [TMA], ethyl [TEA], propyl [TPA] or butyl [TBA]) were mixed with 588 μM Mb or 147 μM Hb, corresponding to equivalent amounts of iron heme (588 μM). After 3 d stirring at ambient temperature, concentrations of free Mb measured by spectroscopy decreased to 3 μM, while the concentrations of free hemoglobin were 0.2–0.8 μM. These results suggested combination of Mb and Hb with the inorganic nanoparticles, which incorporated about 0.6% of the proteins by weight.

Protein–TAAOL-1 dispersions were placed into open-ended capillaries i.d. 1.2–3.0 mm and tilted 45–90°. Samples made with tetralkylammonium ions having methyl (TMA), ethyl (TEA) or propyl (TPA) groups spontaneously formed helices when heated at 85 °C for one week (Fig. 1). In the absence of Mb or Hb, only TAAOL-1 made with TMA spontaneously formed helices. Interactions of Mb and Hb with manganese oxide particles appear to template these helical morphologies.

Protein–TAAOL-1 dispersions were spread onto glass slides and evaporated. The slides were heated at different temperatures for 3 d and then examined by X-ray diffraction (XRD) at room temperature (Table 1). Interlayer distances (*d*) of the composites were typically 13.7 to 18.7 Å between 25 and 85 °C. The materials developed increased interlayer distances (20.0–38.4 Å) between 150 and 180 °C, indicative of Mb and Hb intercalation into TAAOL-1. However, these temperatures are up to 100 °C above denaturation temperatures of the proteins.[10] Mb (25 × 35 × 45 Å) and Hb (50 × 55 × 65 Å) are likely to be denatured to random chains in these materials. Amorphous protein–TAAOL-1 was not transformed between 150 and 180 °C, nor was TAAOL-1 without protein. No ordered structures were formed when using TBA ions.

Aged TPAOL colloids (2 weeks) with d = 15.9 Å were treated with Mb at room temperature. After heating on slides at 40 °C, an ordered layered structure formed with d = 43.8 Å,

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Table 1 Interlayer spacings in Å from XRD for colloidal TAAOL-1, Mb-TAAOL-1. and Hb-TAAOL-1 samples heated on glass slides at different temperatures

T/°C	TMAOL	Mb TMAOL	Hb TMAOL	TEAOL	Mb TEAOL	Hb TEAOL	TPAOL	Mb TPAOL	Hb TPAOL
RT	16.5	13.7	14.1	17.7	am	am	15.5	am	am
40	18.7	13.7	13.9	17.7	17.5	19.7	16.3	13.2	am
70	18.7	13.7	13.3	17.7	17.5	18.3	16.3	13.2	16.8
85	18.7	13.7	13.3	17.7	16.0	18.3	16.3	13.2	16.8
150	13.8	38.4	32.1	9.5	23.8	23.1	am	25.8	34.4
180	9.8	38.4	32.1	am	22.4	19.5	am	23.6	20.0
200	am	am	am	am	am	am	am	am	am

RT = ambient temperature. am = amorphous.

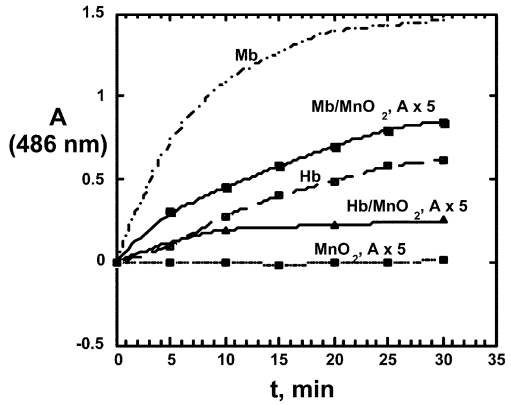

Fig. 3 Hydrogen peroxide (*ca.* 2 mM) activated peroxidase activity for oxidation of *o*-methoxyphenol to product absorbing at 486 nm for TMAOL-1 with and without Mb or Hb (A × 5) compared to activity of solutions of Hb and Mb (A × 1) at equivalent amounts of heme iron.

corresponding to intercalated globular Mb. Between 70 and 85 °C, in the denaturation range for Mb, *d* decreased to 25.9–24.6 Å suggesting collapse of the Mb to a denatured random coil. At 150 °C, *d* remained at 24.6 Å, similar to that of fresh TPAOL-1 combined with Mb. All protein–TAAOL particles became amorphous at 200–250 °C.

The presence of Mb and Hb in the protein–TAAOL-1 particles was also suggested by IR spectra. Most Mb and Hb absorption bands[11] in protein–TAAOL-1 were at similar positions to free proteins. The conformationally informative amide I and II vibration envelopes of the polypeptide backbones[12] were centered at 1654 and 1567 cm^{-1}, respectively, but were overlapped with TMAOL-1 bands. Nevertheless, large intensity increases in this frequency range for protein–TAAOL-1 compared to TAAOL-1 without protein suggested the presence of intact protein in the former material.

Optical microscopy of films of protein–TAAOL-1 on glass slides showed rows of spots in 'finger print-like' morphologies (Fig. 2). SEM analyses (not shown) confirmed film thicknesses of 1.5–5.0 μm.

Hydrogen peroxide activates Mb and Hb for catalytic oxidation of *o*-methoxyphenol, generating colored tetrameric products with absorbance maxima at 470 nm.[8] When hydrogen peroxide (> 1.5 mM) was added to protein–TMAOL-1 dispersions (100 mL) containing *o*-methoxyphenol (5 μg), a new absorption band at 486 nm grew with time, suggesting that tetrameric products of *o*-methoxyphenol oxidation were generated (Fig. 3). The band shift from 470 nm to 486 nm is caused by overlap with the broad tail of the TAAOL-1 at 330–400 nm.[2,3] Results showed that Mb and Hb in the colloidal TAAOL composites retain enzyme-like peroxidase activity. Reaction rates were about 10% of free Mb and Hb. The activity persisted unchanged for Mb and Hb composites that were stored at ambient temperature for one month.

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