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28. This work was supported in part by National Science Foundation grant DMR-9202872, Office of Naval Research grant N00014-91-J-1499, Unocal Corporation, and the University of Missouri Research Board.

21 January 1994; accepted 31 March 1994

Crystal Tectonics: Construction of Reticulated Calcium Phosphate Frameworks in Bicontinuous Reverse Microemulsions

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The chemical construction of organized architectures is an important aspect of innovative materials synthesis. Bicontinuous water-filled microemulsions can be used as preorganized systems for the fabrication of crystalline calcium phosphate materials with extended reticulated microstructures. These macroporous materials are formed by mineralization reactions located within the interconnecting water channels of the bicontinuous network. The resulting materials represent replicas of the microemulsion architecture, but the pore sizes are incommensurate, suggesting that secondary modifications in the bicontinuous microstructure occur during crystal growth. Synthetic macroporous calcium phosphates could have uses in biomaterial implants.

Although the elucidation of "molecular tectonics" (*1*) is still at a fundamental level, it is clear that there are many parallels between the synthetic requirements of materials chemistry and the biological strategies inherent in biomineralization (*2*). Previously, we have shown that organized assemblies of amphiphilic molecules can be used to provide nanoscale environments for inorganic materials synthesis, anisotropic templates for the production of fibrous inorganic-organic composites, and recognition surfaces for the oriented nucleation of inorganic crystals (*3*). Although this approach has been successful in mimicking aspects of biomineralization involving discrete building units, it has not been forthcoming in fabricating elaborate three-dimensional extended architectures analogous to the tests and shells of biological materials such as diatoms and sea urchins. The technological importance of such a relation is exemplified in the recent discovery involving the use of liquid-crystal surfactant aggregates in the control of the pore size of amorphous aluminosilicate materials formed from aqueous solution (*4*). Here, we report an approach based on "crystal tectonics," in which the nanoscale interconnecting rod-like water conduits of bicontinuous water-filled microemulsions act as an organized medium for the construction of an extended macroporous calcium phosphate material. Our approach differs from previous studies with liquid-crystal aggregates in that aqueous compartments are used and reticulated frameworks comprising crystalline inorganic units are fabricated. Moreover, because the bicontinuous network microstructure rapidly fluctuates in liquid media, we have used frozen oils to generate immobilized frameworks for the construction of inorganic architectures.

Bicontinuous reverse microemulsions (*5*) were prepared from mixtures of the cationic surfactant didodecyldimethyl ammonium bromide (DDAB), a metastable calcium phosphate aqueous solution, and a long chain alkane (*6*). The clear mixture was rapidly frozen in liquid nitrogen (*7*) and stored for up to several weeks at temperatures (−25°C for dodecane, +2°C for tetradecane, and −25°C or +2°C for tetradecane-hexadecane mixtures) at which the alkane oils remained frozen (*8*) but the aqueous phase was liquid (*9*). Extraction of the inorganic component formed by mineralization in the aqueous conduits was achieved by centrifugation of the melted microemulsion at 10,000 rpm for 10 to 15 min, followed by extensive washing of the pellet in hot hexane to remove residual surfactant and oil. Depending on the volumes of metastable calcium phosphate used, product yields of 0.5 to 1.0 g per liter of microemulsion were obtained. Samples were subsequently air-dried and studied by x-ray diffraction, infrared spectroscopy, and electron microscopy (*10*).

Scanning electron micrographs of the extracted mineralized replicas showed the presence of highly reticulated microstructures of interconnecting needle-like crystals (Fig. 1). These architectures were observed in all samples prepared at −25° or +2°C. Infrared spectra, electron and x-ray diffraction patterns, and energy dispersive x-ray analysis spectra were consistent with the crystalline mineral hydroxyapatite [HAP: $Ca_{10}(OH)_2(PO_4)_6$]. The majority of materials studied were macroporous with pore diameters up to several micrometers and wall thicknesses of 50 to 130 nm, depending on the storage time and composition of the microemulsion mixture (Table 1). No evidence of regular interconnecting arrays was observed; in all structures the framework appeared to consist of a randomly arranged network of intergrown crystalline needles (lengths of 0.2 to 1 μm) with a wide range of pore diameters. The largest pores were often formed by curved cylinders of the reticulated material and were possibly artifacts of the washing and drying processes. Some modifications in microstruc-

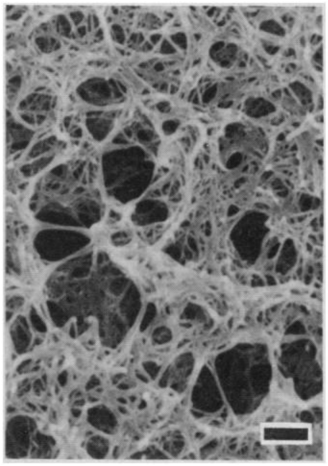

Fig. 1. Scanning electron micrograph showing reticulated framework of needle-like hydroxyapatite crystals formed in bicontinuous microemulsions. The material was prepared from a DDAB-water-tetradecane-hexadecane system at +2°C for 3 weeks. Scale bar, 1.0 μm.

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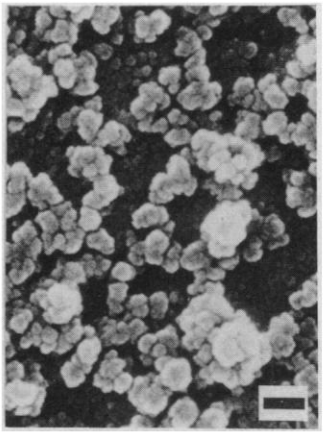

Fig. 2. Scanning electron micrograph of hydroxyapatite synthesized after 2 weeks in DDAB microemulsions at the edge of the bicontinuous phase domain. Note the spherical mineral particles and the absence of a well-defined reticulated architecture. Scale bar, 0.2 μm.

Table 1. Mineralization of reticulated frameworks in bicontinuous microemulsions.

Alkane	Oil (%)	H_2O (%)	DDAB (%)	Storage temp. (°C)	Storage time (days)	Pore size (μm)	Wall size (nm)	Construction unit
C_{14} C_{16}	17 8	35	40	2	21	0.5 to 2.0	50 to 130	Single and bent crystal needles
C_{14}	45	30	25	2	14	0.1 to 2.5	100 to 250	Bundles of crystal needles
	25	50	25	2	14	0.1 to 5.0	100 to 250	
	15	60	25	2	14	0.1 to 8.0	100 to 250	
C_{12}	40	20	40	−25	21	0.04 to 0.7	50 to 130	Single-crystal needles
	55	30	15	−25	14	0.1 to 0.5	150	Spherical crystals
C_{14}	34	50	16 ($C_{12}E_5$)	46	3	0.05 to 1.0	—	Crystal plates

ture were observed with changes in composition. For example, at constant DDAB concentration (25 weight %) increases in the water to tetradecane ratio resulted in larger pore sizes and thicker walls composed of crystal bundles (Table 1). More striking changes in architecture were induced by the preparation of calcium phosphates from microemulsion compositions close to the edge of the single phase region (for example, 55 weight % C_{12}) (Table 1). Under these conditions, the mineralized material was composed of bead-like arrangements of interconnected spherical calcium phosphate particles (Fig. 2). This composition is consistent with the theoretical description of DDAB bicontinuous microemulsions containing alkane oils capable of penetrating the surfactant tails (*11*). In such systems, the rod-like water conduits begin to disconnect into spherical micelles before macroscopic phase separation (*11*).

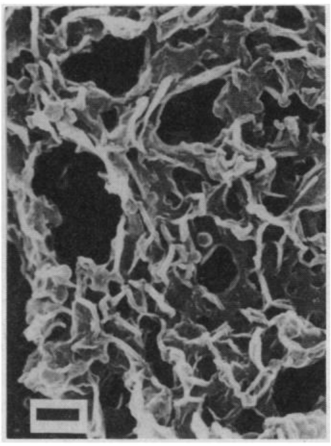

Fig. 3. Hydroxyapatite framework prepared in bicontinuous microemulsions containing the nonionic surfactant pentaethylene glycol dodecylether (46°C for 3 days). The architecture is based on the interlinking of hydroxyapatite sheets. Scale bar, 0.3 μm.

The ability to modify the reticulated inorganic microstructure by secondary changes in the architecture of the associated bicontinuous phase was borne out by the synthesis of calcium phosphate materials in the presence of bicontinuous reverse microemulsions containing the nonionic surfactant pentaethylene glycol dodecylether [$C_{12}H_{25}(OCH_2CH_2)_5OH$ or $C_{12}E_5$] (Table 1). Scanning electron micrographs (Fig. 3) showed the presence of a reticulated material consisting of a network of interconnected mineralized plates, in accordance with the envisaged lamellar structure of the associated bicontinuous microemulsion (*12*).

A striking feature of the reticulated microstructures is that the wall diameters (about 80 nm) are incommensurate with the 1-nm-diameter water channels considered to be present in bicontinuous microemulsions (*13*). Furthermore, the nanometer spacing between branches in the DDAB bicontinuous structure (*13*) is also inconsistent with the micrometer-sized pore diameters observed in the mineralized frameworks. To elucidate aspects of the growth mechanism that could be responsible for the construction of the macroporous inorganic frameworks, samples of a C_{14}-C_{16} microemulsion system stored at +2° (frozen oil) or +25°C (unfrozen oil) were removed at intervals, washed, and examined by electron microscopy. The unfrozen system, which was highly viscous, gave a reticulated material similar to that from the frozen oil medium except that it comprised straighter crystals and domains of extensive aggregation. In both cases, at the early stages of precipitation (2 to 6 hours at +2°C), filamentous strings of mineral particles approximately 1 nm in diameter were visible by TEM. These images suggested that the native bicontinuous microstructure was intact at this stage. However, these aggregates were not present after 4 to 6 days at +2°C but were replaced by needle-like crystals of HAP (100 to 500 nm in length) in association with 100-nm spherules of amorphous calcium phosphate. At first, the HAP crystals were discrete and unconnected, but further growth resulted in a loosely held interconnecting array of relatively straight needles (Fig. 4). The final stage of framework construction (2 to 3 weeks) was characterized by a continued lengthening and thickening of the crystalline units and an increased curvature of the mineral walls.

The results suggest that the nucleation of calcium phosphate is initially restricted to the interconnecting water conduits of the DDAB bicontinuous reverse microemulsion. However, the subsequent growth of mineral particles within the frozen oil medium appears to induce secondary

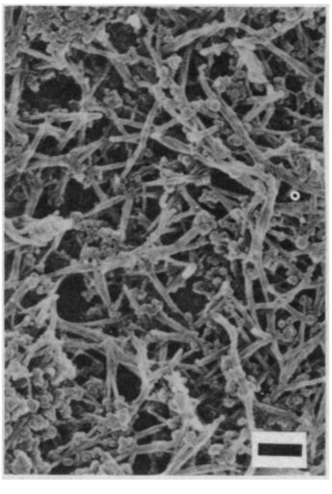

Fig. 4. Scanning electron micrograph of mineralized framework extracted from DDAB bicontinuous microemulsions at an early stage of construction (10 days at 2°C). Note the presence of hydroxyapatite needles and spherules of amorphous calcium phosphate as well as the partially formed reticulated microstructure. Scale bar, 0.5 μm.

changes at the surfactant-water interface. One possibility is that the growing crystals retain a hydration layer together with adsorbed surfactant such that there is a net migration of DDAB molecules toward sites of crystal growth. Therefore, the crystals would no longer be confined to the space delineated by the original water-filled conduits but reshape the microstructure through dynamic changes in the structure of the microemulsion. Although this seems a feasible mechanism in the viscous medium of the liquid oils, it is less obvious that such changes should be associated with microemulsions dispersed in frozen oil. One explanation is that oil molecules associated with the surfactant tails retain some local mobility, although the bulk molecules are frozen. With regard to the construction of the reticulated framework, this construction is probably facilitated by the needle-like morphology of the HAP crystals. This morphology serves to provide effective interlinking and interlocking of the architecture at relatively early stages of formation. Indeed, analogous experiments with CdS, which does not adopt an acicular habit, failed to produce macroporous materials.

Finally, reticulated biominerals, such as corals, have been exploited as biological implants because the macroporosity facilitates intergrowth, vasculization, and the resorption of calcified tissue (*14*). Although the pore size of the reticulated HAP material reported here is possibly too small to allow extensive vasculization, osteoclastic activity and biocompatibility could be expected. In addition, replication of the unperturbed nanoscale microstructure of the DDAB bicontinuous phase might be achieved by minimizing the crystallization forces inherent in systems comprising large crystals such as HAP. Further investigations involving this approach in the synthesis and crystal tectonics of nanoscale cluster materials (for example, CdS) and extended network structures of covalently linked materials (for example, aluminosilicates) are currently being conducted.

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5. The phase behavior and microstructure of disordered open connected bicontinuous microemulsions have been characterized in numerous studies involving conductivity [S. H. Chen *et al.*, *J. Phys. Chem.* **95**, 7427 (1991)], viscosity (F. D. Blum *et al.*, *ibid.* **89**, 711 (1985)], nuclear magnetic resonance spectroscopy [R. Skurveit *et al.*, *J. Colloid Interface Sci.* **152**, 205 (1992)], fluorescence (*11*), and small angle neutron [J. Eastoe *et al.*, *Langmuir* **8**, 1505 (1992)] or x-ray (T. N. Zemb *et al.*, *J. Phys. Chem.* **91**, 3814 (1987)] scattering measurements.
6. A metastable solution of calcium phosphate was prepared by the mixing of equal volumes of a 5.0 mM solution of calcium nitrate (or calcium chloride) with a 3.6 mM solution of potassium dihydrogen phosphate, and the pH was adjusted to 7.4 by 0.1 M NaOH. In the absence of a microemulsion phase, the onset of calcium phosphate precipitation from this solution occurred within 15 min at room temperature. In a typical experiment, 1.5 cm^3 of this solution was added dropwise to 2.5 cm^3 of a rapidly stirred oil-surfactant mixture at room temperature. Bicontinuous microemulsions formed from a range of oil-water-surfactant compositions were studied (Table 1). In each case, the formation of the bicontinuous phase was accompanied by the formation of an optically clear mixture. DDAB [$(C_{12}H_{25})_2(CH_3)_2NBr$] and the alkane oils dodecane ($C_{12}H_{26}$), tetradecane ($C_{14}H_{30}$), and hexadecane ($C_{16}H_{34}$) were obtained from Aldrich Chemical. The oils were filtered through 0.2-μm membranes before use.
7. Rapid cooling in liquid nitrogen was required to minimize phase changes in the microemulsion system that are known to occur between 5° and 15°C.
8. Freezing points are as follows: dodecane, −9.6°C; tetradecane, +5.9°C; hexadecane, +18.1°C. Mixtures of tetradecane and hexadecane were used (hexadecane does not form bicontinuous microemulsions because of poor penetration of the oil into the surfactant alkyl chains).
9. Bicontinuous microemulsions in dodecane, stored at −25°C, were soft in texture, indicating a supercooled aqueous phase.
10. A few milligrams of extensively washed and dried material was examined by x-ray diffraction with a Debye-Scherrer camera and CuK radiation. Infrared spectra of dried samples were studied with the use of KBr disks with a Nicolet Fourier transform-infrared spectrometer. Samples for scanning electron microscopy and transmission electron microscopy (TEM) were prepared by the mounting of small amounts of the mineralized material extracted after centrifugation and careful washing of the TEM grids with hot hexane followed by air drying. Samples observed by SEM were gold-coated and examined with a Jeol 1200EX electron microscope operating at 120 kV Transmission electron microscopy examination. was conducted with Jeol 1200EX and 2000FX electron microscopes operating at 120 and 200 kV, respectively.
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15. We thank Unilever Research for financial support and T. Douglas for advice with experimental work concerning cadmium sulphide.

14 January 1994; accepted 25 April 1994

The Structural Basis of Sequence-Independent Peptide Binding by OppA Protein

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Specific protein-ligand interactions are critical for cellular function, and most proteins select their partners with sharp discrimination. However, the oligopeptide-binding protein of *Salmonella typhimurium* (OppA) binds peptides of two to five amino acid residues without regard to sequence. The crystal structure of OppA reveals a three-domain organization, unlike other periplasmic binding proteins. In OppA-peptide complexes, the ligands are completely enclosed in the protein interior, a mode of binding that normally imposes tight specificity. The protein fulfills the hydrogen bonding and electrostatic potential of the ligand main chain and accommodates the peptide side chains in voluminous hydrated cavities.

In bacteria, periplasmic substrate-binding proteins capture extracellular nutrients and deliver them to membrane-associated complexes that translocate the ligand across the inner membrane and into the cytoplasm (*1*). Several periplasmic binding proteins specific for substrates, such as sulfate, leucine, and maltose, have been characterized in detail. Each consists of two structurally similar domains that engulf the ligand, sequestering it from bulk solvent, by a mechanism that has been likened to a Venus flytrap (*2*). The oligopeptide-binding protein OppA is distinguished from these binding proteins by its broad specificity. It binds peptides (dissociation constant $K_d \approx 10^{-6}$ M) that vary in length from two to five amino acid residues essentially without regard to their side chains; it therefore has many potential ligands, including cell wall peptides of unusual composition and peptide-based antibiotics, yet it excludes single amino acids and related compounds (*3*, *4*). The accommodation of heterogeneous ligands, varying in size, charge, and polarity, within the interior of a protein presents structural and chemical challenges as the stable

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