

Ion-carrier controlled precipitation of calcium phosphate in giant ABA triblock copolymer vesicles

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An ionophore assisted metal-ion transport across block copolymer membranes has been used to control the local Ca^{2+} concentration during precipitation of calcium phosphate in giant block copolymer vesicles.

The controlled formation of inorganic minerals within organic or polymeric matrices is successfully used by nature to design biological composite materials such as bone or teeth.¹ Many of the mineralized tissues formed by organisms have superior mechanical properties. Therefore it has been tempting for a long time to mimic their design principles during fabrication of synthetic (so-called 'biomimetic') materials. Although the detailed mechanisms of biomineralization are still not clarified various models have been suggested that increase our understanding of these biological processes.² A key step in the control of mineralization employed by almost all organisms is an initial allotment of space. This is usually achieved by cellular membranes, predeposited macromolecular matrix frameworks or vesicles. Subsequently minerals are precipitated under controlled conditions within the individual compartments. Vesicular structures are one of the most investigated *in vitro* templates in biomimetic mineralization. Furthermore they also play a crucial role in biological processes such as epiphyseal cartilage or embryonic bone growth.¹ Phospholipid vesicles have been successfully used to mineralize and precipitate inorganic solids, such as *e.g.* calcium phosphate³ or iron oxide⁴ within their interior.

Ion transport across the membranes of lipid vesicles can be controlled by lipophilic ion carriers. This allows control of the local ion concentration inside the vesicles during mineralization.⁵ Similar to biological systems, also during biomimetic mineralization, specific interactions between the matrix forming material and the crystal planes of the growing nuclei are of crucial importance⁶ for the structure and morphology of the newly formed minerals. The high diversity of block copolymer chemistry (*i.e.*, the ease to modify their chemical constitution or attach certain functionalities) makes the self assembled superstructures of amphiphilic block copolymers ideally suited as templates for biomimetic mineralization.⁷ Recently we could show that even membrane proteins can be functionally reconstituted in block copolymer membranes.⁸ Similar to nature, such membrane proteins could potentially be used to tune, for example, the interactions between the matrix and minerals or to control the local ion concentration during precipitation in block copolymer vesicles. The latter could be achieved by incorporating ion carriers or specific ion channels or pumps into the membrane. As a preliminary example of this, we report here on ion carrier-assisted precipitation of calcium phosphate in giant poly(2-methyloxazoline)-poly(dimethylsiloxane)-poly(2-methyloxazoline) (PMOXA-PDMS-PMOXA)⁹ triblock copolymer vesicles. In this work, phosphate anions are encapsulated within the vesicles during their formation. Since the Ca^{2+} ions from the external solution are not able to permeate the triblock copolymer membranes, precipitation of calcium phosphate in the vesicle interior requires a

transport system (see Fig. 1 for a schematic representation). As representative model systems we used three different ionophores that should enable selective or unselective calcium transport from the bulk medium into the intravesicular space. Lasalocid A (X537A, from Fluka)¹⁰ and *N,N*-dicyclohexyl-*N',N'*-dioctadecyl-3-oxapentane-1,5-diamide (ETH5234, from Buchs)¹¹ transport cations *via* a carrier mechanism, where the former shows no specific selectivity and the latter is highly selective for calcium ions. Alamethicin (U22324, from Sigma)¹² is a channel forming peptide that transports cations and anions unselectively.

To facilitate observation of the precipitated calcium phosphate we used giant unilamellar PMOXA-PDMS-PMOXA triblock copolymer vesicles prepared by electro-formation. Here, usually a thin film of polymer is phoresed from conductive glasses or adjacent platinum electrodes by an alternating current into the aqueous solution.¹³ To obtain giant vesicles in higher yields we used a slightly modified set-up based on a two-electrode cell that had originally been designed for EPR studies.¹⁴ The cell consists of a helically wound (0.5 mm diameter) gold wire electrode and a 0.5 mm diameter straight platinum wire supported by two Teflon holders. 2 ml (concentration: 10 g L⁻¹ polymer) of a chloroformic PMOXA-PDMS-PMOXA solution was sprayed onto the gold electrode and the polymer formed a thin film which was dried by blowing a stream of nitrogen over the wire for about 1 min. Afterwards the electrodes were immersed in 2.2 ml phosphate buffer (50 mM K₃PO₄, 10 mM Hepes, 10 mM KCl; pH adjusted to 7.4 with KOH). Giant vesicles were prepared by applying an ac voltage of 5 V at a frequency of 10 Hz for 2 h followed by 30 min at 5 V and 5 Hz. Phase contrast microscopy investigations indicated that the resulting dispersion contained giant vesicles with diameters between 1 and 2 μm in a very high density. Subsequently non-encapsulated phosphate ions can be removed by dialysis against buffer solution (10 mM Hepes, 110 mM KCl, pH = 7.4). After addition of 2 ml of a calcium chloride solution (50 mM CaCl₂, 10 mM Hepes, 35 mM KCl; pH adjusted to 7.4 with KOH) the sample was divided into four aliquots of 1 ml. The first aliquot was mixed with 18 μl of a U22324 solution (1.28 mM in ethanol), the second with 18 μl of an X537A solution (0.44 mM in ethanol) and the third with 18 μl of an ETH5234 solution (0.41 mM in ethanol). As a control experiment we added 18 μl

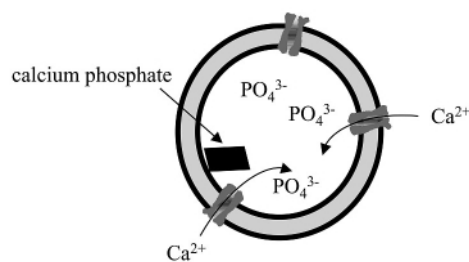


Fig. 1 Schematic representation of ion-carrier controlled precipitation of calcium phosphate in block copolymer vesicles.

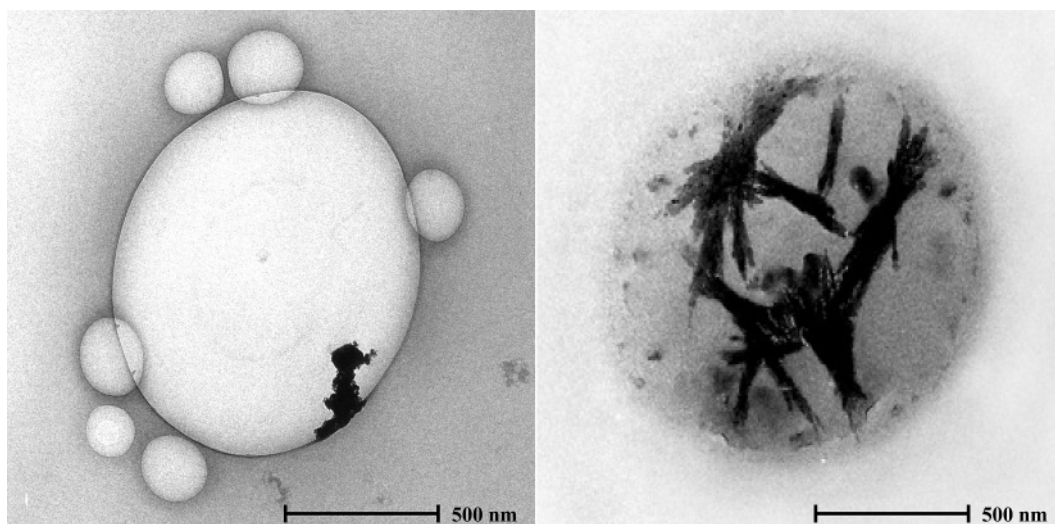


Fig. 2 Transmission electron micrograph of phosphate loaded PMOXA–PDMS–PMOXA triblock copolymer giant vesicles after 1 (left) and 24 h (right) of incubation with CaCl_2 -solution in the presence of the ion carrier ionophore U22324.

of pure ethanol to the fourth aliquot. All samples were incubated at 4 °C and investigated after 1 and 24 h by transmission electron microscopy. To avoid rupturing of the vesicles the samples were kept in a hydrated state during the preparation procedure. In the control experiment without ionophore no precipitation of calcium phosphate occurred. This is in contrast to observations with any of the three used ionophores in which formation of crystals could be observed within the vesicular structures. This clearly shows that the ionophores play a crucial role for the transport of Ca^{2+} ions across the polymer membranes. Fig. 2 shows a representative transmission electron micrograph of phosphate containing triblock copolymer vesicles after 1 and 24 h of incubation with CaCl_2 -solution in the presence of U22324. As can directly be seen already after only 1 h calcium phosphate crystals start to grow at the inner surface of the polymer membrane. After 24 h a considerable fraction of the vesicle interior is filled with needle-like calcium phosphate crystals. Longer incubation times did not lead any detectable further growth of the crystals. Obviously after 24 h the encapsulated phosphate ions have already been consumed. This is also reflected by the fact that the volume of the minerals was always in reasonable agreement with the starting concentration of phosphate in the vesicle. Moreover, the inorganic crystals are clearly confined to the inner cavity of the vesicles thus indicating that crystal growth is limited by the shells of the block copolymer vesicles.

In conclusion, we have successfully applied ionophores to control ion concentration within ABA triblock copolymer vesicles during mineralization of calcium phosphate. Although the ionophores are still rather simple 'vehicles' to transport ions across polymer membranes we believe that the concept of combining artificial block copolymer membranes with natural membrane proteins holds great potential for biomimetic mineralization.

The possibility to incorporate additional design criteria to the block copolymers is straightforward, *e.g.*, functional groups that lead to special surface characteristics of the resulting vesicles

which could be used to control the morphology of the resulting minerals. In this context it is also interesting to note that nature provides many more specific, unspecific and ligand gated channels or pumps (that can additionally be genetically modified!) which can be reconstituted in block copolymer superstructures thus providing a unique tool to control the permeation of ions across polymer membranes. Moreover, the resulting combination of the enormous mechanical stability of block copolymer aggregates with the specificity and efficiency of naturally occurring membrane proteins could even be a helpful tool to get a closer insight into the principles of biomineralization.

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