In situ formation of magnetite nanoparticles inside pores of protein crystals

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Introduction

Properties of magnetite nanoparticles depend of the particle size; therefore, methods able to control particle size are needed[1]. Magnetotactic bacteria, like *Magnetospirillum* strains are able to produce magnetite crystals inside their magnetosomes with a perfect shape and size considered the ideal magnetic nanoparticle[2]. The particular and specie-specific morphologies of those magnetosomes are still not well understood, and some authors have pointed, as one of the possible cause, the confined space in which those crystals are produced. To study the formation of magnetite crystals in a confined space we have created cross-linked protein crystals, that are highly porous materials with a defined pore size, and used them as a matrix to promote the magnetite formation.

Materials & Methods

The three model proteins, lysozyme, lipase and glucose isomerase, were crystallized using batch and handing drop methods[3]. To improve mechanical and chemical stability crystals were cross-linked with glutaraldehyde.

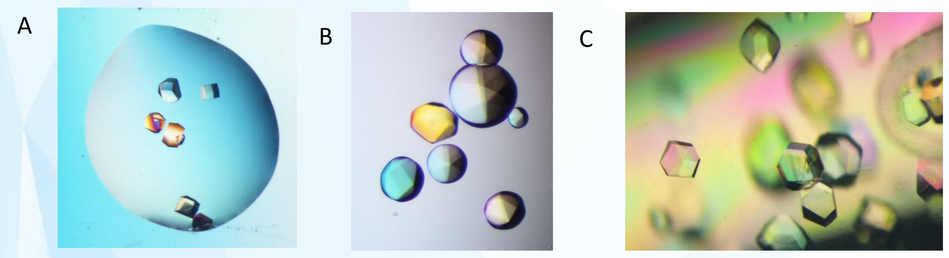

Figure 1. Example of crystals of (A) lysozyme, (B) lipase and (C) glucose isomerase obtained by hanging (A & B) drop and batch (C) methods.

For the in-situ formation of magnetite, cross-linked crystals were soaked with the co-precipitation solution ($Fe(ClO_4)_2$, $FeCl_3$, $NaHCO_3$ and Na_2CO_3) in an oxygen-free atmosphere within the "COY" anaerobic chamber. After incubation, biomineralization of magnetite was trigger by the addition of NaOH[4].

Results and discussion

Transmission electron microscopy (TEM) and high resolution transmission electron microscopy (HR-TEM), confirmed formation of magnetite in the solution where the crystals were incubated (typical diffraction pattern of magnetite) and penetration and accumulation of iron ions inside the pores of lysozyme crystals (by X-ray spectrum of the particles). The absence of diffraction of the latest particles formed inside crystals pores corresponds to the amorphous state. The equilibration of the amorphous state could be explained by the inhibition of magnetite maturation inside protein crystals pores due to confined space or/and interaction between iron ions and charged amino acids located around protein crystals pores.

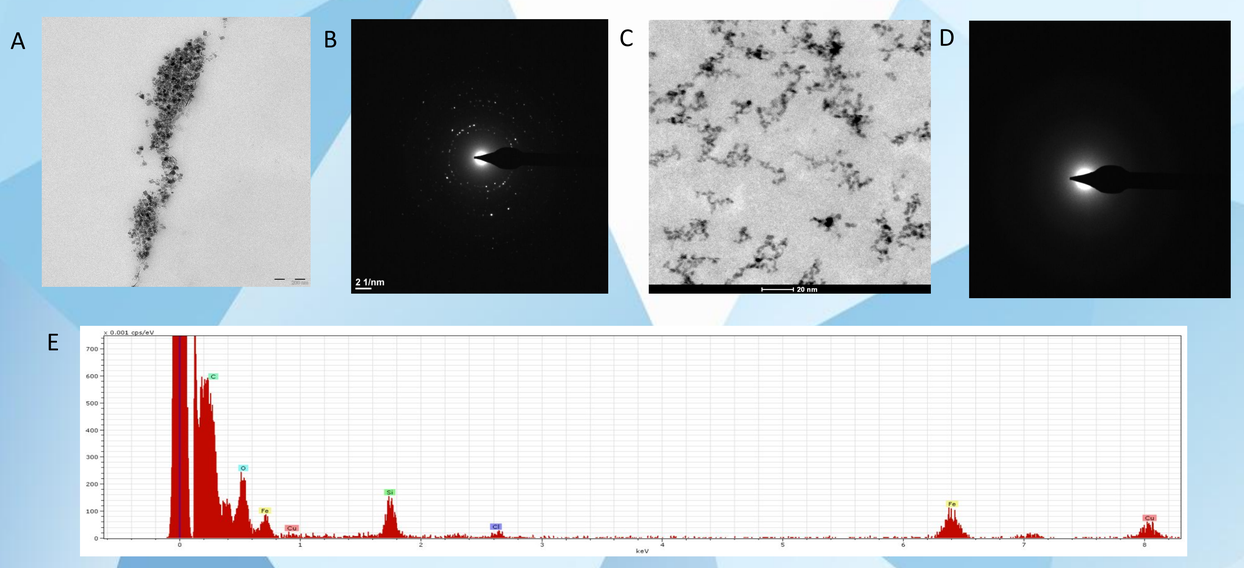

Figure 2. (A) TEM image of magnetite outside of the crystal. (B) The diffraction pattern of magnetite outside of the crystal. (C) HR-TEM image of lysozyme crystals with particles inside. (D) diffraction image of the particles inside the lysozyme crystals. (E) Energy Dispersive X-ray spectrum of the particles inside the lysozyme crystals.

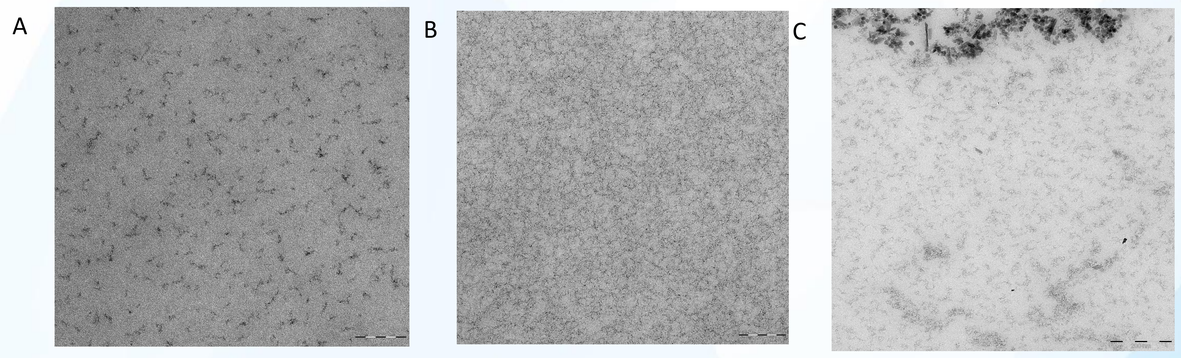

Figure 3. TEM images of (A) lysozyme, (B) lipase, and (C) glucose isomerase crystals with magnetite particles inside.

The particles inside lysozyme, lipase and glucose isomerase crystals have the same size (2-3 nm) despite the fact that lipase and glucose isomerase crystals have bigger pores (around 7nm and 4nm respectively) than lysozyme (around 2 nm).

For evaluation of magnetite evolution and growth through time, crystals were incubated every week in a new solution for biomineralization to avoid exhausting iron ions.
After each week the crystals were studied on TEM.

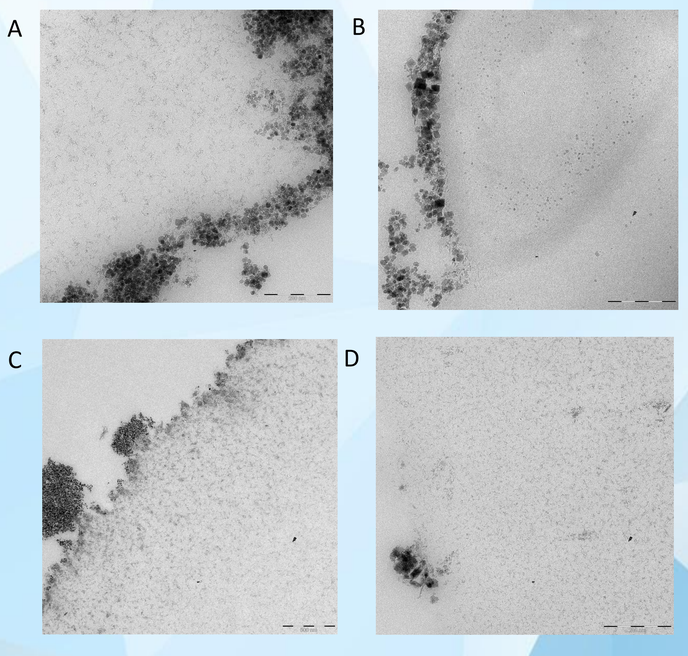

Figure 3. TEM images of glucose isomerase crystals incubated 2 weeks (A) and 6 weeks (B); and lysozyme crystals incubated 2 weeks (C) and 6 weeks (D).

The results have shown that the size of nanoparticles is maintained throughout the time: after 2 and 6 weeks of incubation the average particles size was stable: around 2-3 nm inside both lysozyme and glucose isomerase crystlas. That could be an evidence of confined growth caused by pores of protein crystlas.

Conclusion

Our preliminary data show the formation of small iron nanoparticles inside protein crystals. The size of the nanoparticles is maintained throughout the time and associated with the size of the pores of protein crystals. These results could indicate confined growth caused by the pores. Further studies will give more information about the role of protein crystals in the formation of magnetite nanoparticles.

Acknowledgments:
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