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High concentrations of proteins favors protein-mineral interactions and stabilize the amorphous state of calcium orthophosphate: A solid-state nuclear magnetic resonance (NMR) study of the casein

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Solid state NMR is a remarkable tool to understand how the properties of heterogeneous organic-inorganic composite materials. In the present work, we have used NMR to study the capability of proteins to preserve amorphous calcium phosphate (ACP) phases. Most of the former studies were performed under conditions where ACP colloidal nanoclusters are surrounded by low-to-moderate concentrations of peptides or proteins (15-30 g L^{-1}). Therefore, the issue of ACP-protein interactions in highly concentrated protein systems has clearly been overlooked. In this work, nano clusters of ACP associated to a phosphoprotein network, called casein micelles, are monitored using solid-state NMR under two elevated protein concentration (300 and 400 g L^{-1}). In all concentrations, at 25 °C, it is establish that the proteins preserve the mineral phase in an amorphous structure with no change on nano-cluster size and equilibrium. NMR show that in both concentrations a significant amount of the nonphosphorylated side chains interacts with ACP through hydrogen bonds. However, the number of these interacting side chains is found to be much higher at 400 g L^{-1} than at 300 g L^{-1} indicating that concentration favors this specific interaction in contrast to phosphorilated side chains which do not display any changes. At 45 °C, which is a destabilizing temperature of ACP under protein-free conditions, the ACP nanoclusters are partially transformed 300 g L^{-1}, while it stays almost undamaged at a protein concentration of 400 g L^{-1}. These data clearly point out that increasing the concentration of proteins favors ACP-protein interactions and preserves the ACP nanoclusters more efficiently.

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