Quantitative proteomics provides new insights into chicken eggshell matrix protein functions during pivotal stages of shell mineralization

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Eggshell is a biomineral made of 95% calcium carbonate (calcite) and 3.5% organic matrix (proteins and proteoglycans). This structure is the consequence of controlled interactions between both mineral and organic matrix constituents resulting in a highly ordered structure of the eggshell, with unique mechanical properties. The calcification process occurs in the uterus where biomineralization follows a temporal sequence. The primary events of mineralization phase have been recently defined[1] as 1) the widespread deposition of amorphous calcium carbonate (ACC), 2) ACC transformation into crystalline calcite aggregates, 3) formation of larger calcite crystal units. This initial phase is followed by 4) the development of a columnar structure with preferential calcite crystal orientation during the active calcification growth phase, and 5) the termination of calcification just prior the oviposition. We have used quantitative proteomics on uterine fluid [2] and forming eggshell samples [3] collected at these 5 pivotal stages to explore the distribution and variations of abundance of about 300 eggshell matrix proteins according to these calcification events. We combined quantitative data with emPAI values in order to discern the most abundant proteins at each stage considering that the most active proteins for shell calcification should be present amongst them. We distinguished proteins having a direct involvement in shell mineralisation (mineralising proteins, able to bind calcium or divalent ions) from proteins indirectly related to the calcification process (involved in the regulation of proteins driving mineralisation).

We highlighted 24 matrix proteins that we suspected to have predominant roles in the control of the different stages of shell calcification. Lysozyme and ovotransferrin can interact with calcium as shown in vitro and are present with a high emPAI values at all stages of shell formation. The hypothesis that OC-17 and ovalbumin stabilize ACC was reinforced by the observation that both proteins showed high emPAI values and were overabundant in the shell at the early stages of shell formation. EDIL3, ALB, MFGE8, HPX and NUCB2 are calcium binding proteins and promising candidates to stabilize ACC or to control growth and morphology of calcite crystals. LOXL2 is involved in eggshell membranes formation. OC-116, HAPLN3, SDCBP, TSKU and GPC4 are proteins associated with the proteoglycan family which is thought to influence the CaCO3 biomineralisation process in many species including hen. Polymorphism of OCX32 is associated with shell quality traits and this protein is overabundant in the shell when large columnar calcite crystals develop. CLU, PPIB and OCX21 are three major molecular chaperones possibly involved in the appropriate conformation of the shell matrix template. OVM, CST3, OIH and OCX25 are protease inhibitors which might control the calcification process by degrading or maturing proteins driving shell mineralisation. Finally, LOC428451 corresponds to a phosphatase influencing the degree of phosphorylation, process which has been demonstrated to modulate the kinetics of mineralisation in eggshell. Out of more than 600 proteins revealed by previous proteomic studies of eggshell matrix, we selected these 24 proteins due to their high emPAI values and changes during the different shell formation stages. Characterization of their function in the process of shell mineralisation will require further studies in vitro using purified proteins or in vivo to define their interactions with the mineral phase.

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References

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