

## Inhibition and regulation of calcium carbonate crystallization by protein hydrolysates

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### Summary

Various protein hydrolysates showed inhibitory activities on the crystal growth of calcium carbonate. The activities increased by treating the protein hydrolysates with glutaminase and by the addition of sodium chloride, lactose, etc. Such interactions between calcium ions and organic compounds are important for biomineralization. In fact, the multiple lectins (BRA-2, BRA-3), which are a group of sugar-binding proteins, from the hemolymph of the acorn barnacle (*Megabalanus rosa*) inhibited the crystal growth at a lectin concentration of >0.1 mg/30 ml. A galactose-binding lectin (BRL) was also isolated from the acorn barnacle (*Balanus rostratus*), however, it had no inhibitory activity. Thus, the amino acid sequence of BRL was determined to explore the structure-activity relationship in terms of the inhibition of the crystal growth of calcium carbonate. BRL was composed of identical subunits of 182 amino acids. BRL was 46% identical to BRA-2 and 15% identical to BRA-3. Therefore, BRL and BRA-2 may have evolved from the common ancestor gene. The distinct activities of these lectins may attribute to the number and the localization of acidic amino acids and their amide forms on the surfaces of the lectin molecules, that interact with the growth sites on crystal surfaces.

Observations by scanning electron microscopy revealed modifications of the size and the morphology of the calcium carbonate crystals grown in the presence of various peptides and proteins. In the presence of peptides and proteins, the crystals seemed to be less well crystallized and smaller, and they showed smooth edges unlike the crystals obtained without peptides and proteins. In conclusion, protein hydrolysates play important roles in regulation of crystal growth, including nucleation, rate of growth, orientation, size and overall morphology of crystals.