

研究業績 英文表記

和文	
表題	バイオミネラリゼーション、形成、多様性、進化、適応
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英文	
Title	Biom mineralization (BIOM2001): formation, diversity, evolution and application
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Abstract	<p><i>Lingula</i> is believed to be one of the earliest animal in the earth history with a hydroxyapatite exoskeleton. They are still living in limited areas in Japan such as Ariake Bay. The aim of this study is to comparatively analyze the matrices of hydroxyapatite skeleton of <i>Lingula</i> and higher vertebrates. The major difficulty in biochemical analysis of the <i>Lingula</i> matrix resides in its insolubility. Here we report on a new method of solubilization of the <i>Lingula</i> matrix by applying chitinase and on a collagenous protein with a unique amino acid sequence that was detached in the solubilized fractions. Shells of fresh <i>Lingula unguis</i> were cleaned and crushed in liquid nitrogen to obtain fine powders that were separated into higher (density>2.5) and lower density (density<2.5) fractions in a bromoform-acetone mixture. Both fractions were further processed in two ways. In one method, the powders of the <i>Lingula</i> shell with both densities were decalcified in 0.5 M EDTA, pH 7.4, and then extracted with 4 M guanidine-HCl for analysis of the soluble fraction. In the other method, they were decalcified in 0.01 M HCl, extracted with 6 M urea, and the residues were provided for chitinase digestion. In the 4 M guanidine soluble higher density fraction (mineralized layer), a 39 kDa protein was purified as was a 16 kDa protein from the lower density fraction (unmineralized layer). From the sequence analyses, the 39 kDa protein was revealed to be a novel protein, having a first eleven amino terminal sequence of SNXPVNAAGGV that was not found in the protein database. The residues after 6 M urea extraction were digested with chitinase, dialyzed, lyophilized and chromatographed on a hydroxyapatite column (elution with a 5-400 mM phosphate gradient in 6 M urea). From the first of five distinct peaks, a 43 kDa was purified and found to be a novel protein having a unique repeat of glycine in every third position: GAXGAXGAX--GKKGKKGAX, where the X was assumed to be hydroxyproline. The sequence suggested the presence in the shell of a collagenous protein that was somewhat different from the mammalian type. On the other hand, by transmission electron microscopic observation it was revealed that, in the soft connective tissues in the inner layer adjacent to the shell, there were abundant collagen fibrils with typical striations. The results indicate that collagenous proteins in the <i>Lingula</i> shell may have roles other than those of the soft tissue collagen, probably involved in calcification process.</p>
keyword	<i>Lingula unguis</i> , organic matrix, soluble proteins, chitinase digestion

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