

Identification of cement-like substance connecting silica scales in testate amoeba *Paulinella chromatophora* and its evolution.

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Testate amoebae belonging to Euglyphida (Cercozoa, Rhizaria) have a shell composed of silica scales, which are bonded with cement-like substance each other. Detailed shell construction process had been observed using a euglyphid *Paulinella micropora*, and it had been found the scales are assembled into a shell one by one using the cement-like substance, which thought to be secreted from the dense-material-containing vacuoles located at the front edge of pseudopodium. While the cement-like substance is vital for the shell construction process of testate amoebae, the nature of the cement-like substance has yet to be revealed. Recently we found that soybean agglutinin (SBA-lectin), which specifically recognizes *N*-acetylgalactosamine (GalNAc), binds to the materials between scales of *P. micropora* shell and thus the lectin is thought to be a key to identifying the cement-like substance. To reveal the nature of the cement-like substance of *P. micropora*, in this study we tried to identify the materials between scales, which binds with the SBA-lectin. The materials were purified from whole cell lysate by SBA-lectin affinity column. We detected a 130 kDa band by a lectin blot analysis with the purified sample and then the materials within the band were analysed by LC-MS/MS. The result suggested existence of cellobiose phosphorylase (CBP), a cellobiose phosphorolysis enzyme. Since the CBP of *P. micropora* was predicted to have four *O*-glycosylation sites, the protein could be glycosylated with GalNAc. Phylogenetic analysis of the *P. micropora* CBP protein sequence with those from broad range of organisms showed that CBPs split into two large groups, prokaryotic- and eukaryotic-type and the eukaryotic-type CBP proteins consists of sequences only from stramenopiles, haptophytes and euglyphids. The tree topology suggested that the CBPs of stramenopiles and euglyphids share a common origin.