C1q Domain Interactions in Otolith Morphogenesis

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Abstract

Otoliths ("ear stones") in fish and its homologous otocionia ("ear dust") in higher vertebrates are essential in detecting linear acceleration, as well as providing a sense of balance. They consist of mainly CaCO3 which interweaves with proteins to form a matrix. The investigation of otolith morphogenesis allows proteins forming the matrix to be identified and characterized. The recently identified precerebellin-like protein (Cblnl) in trout otolith has a C1q domain that is capable of interacting with the C1q domain of Otolin-1 (Otol1a) in zebrafish otolith. It is proposed that the C1q domain of Otol1a forms heteromers, and heteromers with that of Cblnl. By performing site-directed mutagenesis, we successfully deleted the two introns in trout Cblnl genomic clone to create a cDNA, which is now available for subcloning into a bacterial expression vector. We then expressed the recombinant Otol1a C1q domain via pET system and carried out affinity purification under denaturing conditions. Isolated target proteins were then refolded by dialysis in urea step gradient buffers and a fractional matrix with various reagents at different concentrations.

Introduction

Otoliths ("ear stones") in the inner ear of zebrafish are homologous to the otocionia of mammals, playing significant roles in linear acceleration detection and balancing. They consist of a meshwork of proteins interspersed with CaCO3 crystals. The secretion and assembly of the organic matrix into the endolymphe provides a template for biomineralization, thereby initiating otolith calcification in daily ring-like increments.1 Collagens – large domains of repeating Gly-X-Y sequences folded into a triple-helical structure – are present in Otolin-1 (Otol1a), a type of matrix protein in zebrafish otolith. These collagens in Otol1a are related to the Collagen VIII and X family and are essential for the structural organization of the matrix prior to calcification via multimer assembly. They exist in association with a C1q domain, which is also found in the Precerebellin-like protein (Cblnl) in trout otolith; however, instead of the collagen-like region, Cblnl has a homologous NC1(X) domain that is capable of trimeric or higher order multimer assembly without adjacent triple helical segments. Hence, we propose that the C1q domain of Otol1a forms homomers and interact with that of Cblnl during otolith framework assembly. Trimers of C1q domain forms hubs while collagen fibrils (triple helices) form spines, assembling the short chain collagens into a reticular network of matrix.2

A Collagen Framework in Otolith Formation?


Materials and Methods

• Deleting the two introns in trout Cblnl genomic clone to create a cDNA
• Express Otol1a C1q domain and affinity-purify under denaturing conditions and decreasing pH
• Assess solubilization and refolding of Otol1a C1q domain.

Results

Figure 1 shows the experimental process.

Figure 3 shows Coomassie stained SDS-PAGE image of column affinity purification of Otol1a C1q domain. Lanes 2&17: size markers (key at right panel); Lane 1: total cell protein; Lane 3: cell lysate; Lane 4: Flow-through; Lane 5: Wash at pH 8.0; Lane 6: Wash at pH 6.8; Lanes 7-16: Fractions 7-16 eluted with pH gradient (6.8-4.5) followed by pH 4.5. Fractions 7-16 showed Otol1a C1q domain of expected size (~15 kDa), present in all fractions due to overexpression. Fraction 14 showed the most dense band indicating the highest concentration of C1q domain, along with additional bands at ~30 kDa and ~50 kDa, suggesting the presence of dimers and trimers.

Figure 4: Chromatogram of column affinity purification of Otol1a C1q domain during day 1 and 2. with fractions indicated. Absorbance plateaued during sample load due to high amounts of overexpressed proteins. Day 2 showed sharp increase in absorbance at fraction 14 during elution at pH 4.5.

Conclusions

• The two introns in trout genomic DNA were successfully deleted.
• Otol1a C1q domain was expressed and purified with high yield (~40 mg) and purity (~95%).
• The denatured Otol1a C1q domain was solubilized and possibly refolded in the presence of arginine.

Future Directions

• Subcloning of open reading frames into bacterial expression vector for Cblnl C1q domain, followed by expression and purification.
• Test additional adjuvants for refolding and access multimerization of Cblnl and Otol1a C1q domain.

Literature Cited


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