
Introduction Cartilage oligomeric matrix protein (COMP) is a member of the thrombospondin (TSP) family of extracellular glycoproteins found abundantly in cartilage. COMP polypeptide chains are composed of a linear array of domains that include an oligomerization site that forms a coiled-coil structure, 4 epidermal growth factor repeats (EGF), the TSP type III region, and a C-terminal globular domain. Mutations in COMP are associated with pseudoachondroplasia (PSACH) and multiple epiphyseal dysplasia (MED) and occur predominantly within the TSP type III domains, the region proposed to bind calcium. Because the majority of PSACH-associated mutations of COMP occur within the putative calcium binding region, it seems likely that the binding properties of mutant peptides may be affected. A previously described mutation, Asp446Asn, demonstrated that COMP was sequestered inside the endoplasmic reticulum (ER) vesicles of PSACH chondrocytes (1). To better understand the pathogenesis of the distinctive ER vesicles associated with COMP mutations, the region encompassing the Asp446Asn mutation was expressed in a recombinant cell culture system. The structural features of this mutant peptide are compared with a wild-type peptide and provide evidence for reduced functional properties associated with poor calcium binding.

Methods Cartilage from the iliac crest of a patient with PSACH was removed during elective surgery with the informed consent of the patient and his family. Total RNA was isolated from cultured PSACH patient chondrocytes and used as a template in PCR amplification using primers to the TSP III region: sense (5-actagtttatgtgccatcagatg-3); antisense (3-ccpgagaagctagctggtcgcgacactgacctagac-5). Control RNA was likewise prepared. One wild-type and one mutant transcript was ligated into the expression vector pCEP that had previously been engineered for efficient secretion of extracellular proteins; the expression construct was used to transfect 293-EBNA. Following selective culture conditions, cell culture medium was tested for the presence of COMP peptides. Medium from the transfected 293 cells was collected and fractionated with ammonium sulfate and the COMP-3 peptides purified using ion-exchange and size-exclusion chromatography. Circular dichroism (CD) spectra of the COMP peptides in 10 mM MOPS pH 6.8 was analyzed in the presence and absence of 5 mM calcium chloride. Calcium binding experiments were carried out using Dispo-dialyzer units (The Next Group). COMP3 peptide solutions were dialyzed against concentrations of calcium chloride ranging from 50 µM to 2 mM containing a small amount of radiolabeled calcium was added for quantitative purposes. Aliquots from each chamber were counted and the values for bound versus free calcium determined for the relative concentration of protein. The data was analyzed using a least-squares statistical method and the results from the wild-type and mutant peptides compared.

Results Differences in secondary structure between the wild-type and mutant COMP-3 peptides was observed by analysis of the circular dichroism spectra, notably the loss of helical structure and an increase in the antiparallel beta sheets in mutant form. Calcium binding by equilibrium dialysis revealed that the mutant peptides were able to bind less than half of the wild-type amount. The free and protein-bound calcium concentrations were calculated for several experiments using protein concentrations that varied from 1.6 to 10.3 µM (Fig. 2). The P-value, a measure of the cooperativity of the binding curve, showed COMP3-Wt bound calcium in a more cooperative manner.

Discussion This study provides initial evidence that COMP mutations associated with PSACH alter the structure and functional properties of the affected region of the molecule. It may be presumed that the type III repeats of COMP bind calcium in a similar way to thrombospondin I, where it has been shown that up to 12 calcium ions may bind a single polypeptide chain (2). The data presented here fit the predicted number of binding sites, although a slightly higher number of ions per peptide are indicated. What is dramatic here is the reduced calcium binding ability of the mutant peptides. The effect of that mutation is further indicated in the structural studies that show a much smaller change in shape in the presence of calcium. Because the mutant peptide contains only one amino acid change and yet binds less than half of the normal amount of calcium, it seems likely that other factors are involved. Calcium binding to TSP I occurs by a cooperative mechanism, implying that there is a conformational change upon initial binding that effects the conformation of other binding sites. The impact of an amino acid mutation at one calcium binding site may alter the binding at other sites by at least slowing the process of calcium binding if not actually preventing further binding. The downstream consequences of poor calcium binding in mutant COMP peptides requires further studies. These will be instrumental in understanding the molecular mechanisms involved in the skeletal disease process.