Characterization of osteoadherin—a novel, cell binding keratan sulfate proteoglycan from bone

Mikael Wendel, Yngve Sommarin and Dick Heinegård

Department of Cell and Molecular biology, University of Lund, P.O. Box 94, S-221 00 Lund, Sweden.
Tel +46-46-222 85 99. Fax +46-46-211 34 17.

The extracellular matrix of bone is mineralized with crystals of hydroxyapatite. The spatial orientation of the mineral crystals are dependent on the most abundant bone matrix protein, type I collagen. Bone also contains a number of non-collagenous proteins that appear to have important roles in various aspects of tissue homeostasis (Heinegård and Oldberg 1993, Young et al. 1992). During the last two decades several proteins have been isolated from bone tissue and characterized. Much interest has been focused on osteocalcin, matrix gla-protein, osteonectin, osteopontin, bone sialoprotein, and the small proteoglycans; decorin and biglycan. Still, in most cases very little is known about their functions in the tissue.

We have identified, isolated, characterized and determined the primary structure of a predominating small proteoglycan (PG) with high affinity for hydroxyapatite from bovine bone.

Results

The PG was first noticed as a component in guanidine hydrochloride/EDTA extracts of bovine bone. This proteoglycan was purified to homogeneity in three chromatographic steps. First, chromatography on DEAE-cellulose in 7 M urea, 0,1 M sodium acetate pH 6, followed by chromatography on hydroxyapatite in 7 M Urea, 20 mM sodium phosphate, pH 8. Final purification was achieved by gel filtration, in 4 M guanidine-HCl, on Superdex 200. The reduced protein gives a single band of 85 kDa apparent molecular weight on silver stained SDS-polyacrylamide gels, indicating high purity.

Digestion of the 85 kDa PG with N-glycosidase reduced the apparent size of the protein to 47 kDa, showing the presence of several N-linked oligosaccharides. Digestion with keratanase indicated that some of these oligosaccharides are extended to keratan sulfate chains.

An antiserum was prepared and was shown to be specific for the 85 kDa proteoglycan in western blots of total bone extracts. Metabolic labeling of primary bovine osteoblasts followed by immunoprecipitation shows that the cells synthesize and secrete the protein.

The PG has cell-binding functions since it promotes osteoblast attachment in vitro. The cell binding efficiency is comparable to that of fibronectin (unpublished observations).

The primary structure of the 85 kDa PG was determined from a cDNA-clone isolated from a bovine primary osteoblast expression library by antibody screening. The identity of the clone was confirmed by amino acid sequences from eight tryptic peptides. The bovine cDNA encodes a protein of 49 116 Da, including a 22 amino acid long signal peptide. About two thirds of the 85 kDa proteoglycan consists of eleven leucine-rich repeats (LRR). Disulfide bonded domains are flanking the repeat region on both the N- and C-terminal end. Six consensus N-glycosylation sites and 3–4 tyrosine sulfation sites are present.

The presence of leucine rich repeats shows that the 85 kDa proteoglycan belongs to the family of small proteoglycans. The highest similarity is to fibromodulin and lumican, with 36% and 38% identical residues, respectively. The identity to the two other leucine rich repeat containing bone proteoglycans decorin and biglycan is 25% and 26%, respectively.

Discussion

The isolation and characterization of novel macromolecular constituents of bone provides important tools for studying bone biology. We have purified and determined the primary structure of a 85 kDa keratan sulfate proteoglycan from bovine bone. The proteins belongs to the family of leucine rich repeats. It has cellbinding capacity and may be a link between cells and the mineralized matrix. Another potential role for the 85 kDa PG by analogy with other members of the LRR-family, are to sequester growth factors in the extracellular matrix.

We propose the name osteoadherin in view of the high affinity to hydroxyapatite and the cell-binding activity.

References

1. Heinegård D, Oldberg Å. Glycosylated matrix proteins. In: Connective Tissue and Its Heritable Disorders (Eds. Royce and Steinmann). Wiley-Liss, Inc. New York 1993: 189–209.
2. Young M F, Kerr J M, Ibaraki K, Heegaard A M, Robey P G. Structure, expression, and regulation of the major noncollagenous matrix proteins of bone. Clin Orthop 1992; 281: 275–94.