Molecular cloning, recombinant expression, and purification of osteocalcin in sika deer (Cervus nippon) antler

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Abstract

Osteocalcin is a major non-collagenous component of the bone extracellular matrix and is considered to be an indicative factor of osteoblast differentiation. In the present study, we detected osteocalcin expression in different antler areas and growth phases by immunohistochemistry. Osteocalcin was highly expressed in all areas during the mineralization period and in mesenchymal cell and chondrocyte areas during the rapid growth period. The nucleotide sequence of the osteocalcin gene in sika deer antler was determined. The open reading frame was 303 bp encoding a protein of 100 amino acids. The estimated molecular mass of osteocalcin was 10.38 kDa and the theoretical isoelectric point was 5.37. The osteocalcin gene with a 6× His-tag at the C-terminus was cloned into the pGEX-4T1 vector and expressed in Escherichia coli under optimal conditions. The recombinant soluble protein fused with GST was purified with Ni-NTA resin. The purified osteocalcin protein exhibited a significant increase in HA adhesion and promoted antler chondrocyte proliferation. Osteocalcin is an important factor in regulating the rapid growth and differentiation of deer antlers.

Key words: Cervus nippon, osteocalcin, molecular cloning, expression, purification

Introduction

Osteocalcin, also known as bone Gla protein, is synthesized and secreted exclusively by osteoblasts and osteocytes at the late stage of maturation. This protein is a major non-collagenous component of the bone extracellular matrix and is considered to be an indicative factor of osteoblast differentiation (Huang et al. 2016, Li et al. 2016). Osteocalcin undergoes post-translational modification whereby available glutamic acid residues are γ-carboxylated (Hauschka et al. 1975, Price et al. 1976). Mature osteocalcin is secreted into the skeletal microenvironment and then goes through a conformational change that aligns its calcium-binding Gla residues with the calcium ions in hydroxyapatite. This property was initially proposed as a mechanism that enables osteocalcin to initiate the formation of hydroxyapatite (HA) crystals (Ducy et al. 2011). There are two forms of osteocalcin in serum: carboxylated and uncarboxylated (Hauschka et al. 1989).