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Review

Dentin sialophosphoprotein-derived proteins in porcine pulp and dentin – Gene expression and function

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ABSTRACT

Background: Dentin sialophosphoprotein (DSPP) is the most abundant non-collagenous protein in dentin and is critical for the proper mineralization of tooth dentin. DSPP is processed by proteases into three major domains: dentin sialoprotein (DSP), dentin glycoprotein (DGP) and dentin phosphoprotein (DPP). Two mRNA variants are expressed from the *Dspp* gene. The larger transcript encodes full-length DSPP (DSP+DGP+DPP). The shorter transcript encodes only DSP.

Highlight: We fractionated DSPP-derived proteins from the dental pulp of developing porcine incisors using heparin chromatography. DSP was identified, but little DPP could be detected in any fraction. Expression of full-length *Dspp* mRNA, determined by qPCR analysis, was significantly higher in odontoblasts than in pulp. Expression of DSP-only mRNA was almost equal in odontoblasts and in the body of pulp. Expression of full-length *Dspp* mRNA was also significantly higher than expression of DSP-only mRNA in odontoblasts. Both the full-length and DSP-only *Dspp* mRNA showed only trace expression in the pulp tip. We purified TGF-β1-unbound or -bound to DPP and DSP using high performance liquid chromatography (HPLC) and measured its alkaline phosphatase stimulating activity in human periodontal cells with or without TGF-β receptor inhibitor. We also incubated carrier-free human recombinant TGF-β1 (CF-hTGF-β1) protein with TGF-β1-unbound DPP or DSP and characterized binding ability.

Conclusion: DSP-only is expressed throughout odontoblast differentiation, while full-length DSPP is predominantly expressed by odontoblasts only after they have differentiated from mesenchymal cells. DPP and DSP rescued the loss of TGF-β1 activity. Type I collagen was infrequently bound to CF-hTGF-β1.

Contents

Abbreviations: ALP, alkaline phosphatase; BMP, bone morphogenic protein; ELISA, enzyme-linked immunosorbent assay; HPLC, high performance liquid chromatography; MMP, matrix metalloproteinase; qPCR, quantitative polymerase chain reaction; RT-PCR, reverse transcription-polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TGF-β, transforming growth factor-beta

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1. Introduction

Dentin is the predominant mineralized tissue comprising the body of a tooth. Collagen constitutes approximately 90% of the dentin organic matrix [1]. The non-collagenous proteins in dentin are dominated by DSPP-derived proteins, which are generated by proteolysis of dentin sialophosphoprotein (DSPP) [2]. Porcine DSPP is expressed and secreted by odontoblasts and is processed by BMP-1, MMP-20, and MMP-2 [3,4] into three main components: dentin sialoprotein (DSP) [5,6], dentin glycoprotein (DGP) [7], and dentin phosphoprotein (DPP) [8,9].

Dental pulp is a connective tissue derived from the dental papilla. The differentiation of odontoblasts from the dental papilla is caused by the expression of signaling molecules, transcription factors, and growth factors by cells of the inner dental epithelium [10]. DSPP has been used as a marker for the *in vitro* differentiation of dental pulp-derived stem cells into odontoblasts [11–13]. In immunohistochemical and *in situ* hybridization studies in mice, DSP protein and mRNA levels increase, coinciding with the progression of odontoblast differentiation [14].

In addition to DSPP-derived proteins in pulp, this bioactive component has also been found in the extracellular matrix of dentin [15]. This inductive activity was inhibited by the neutralizing TGF-β antibody [15]. TGF-β isoforms have been extracted from dentin matrices of both rabbit and human teeth [16]. TGF-β1 is the predominant isoform with approximately half of that present in the active form [17]. Dentin matrix sequesters TGF-β proteins and acts as a reservoir for its activity. Decorin and biglycan have been proposed as potential TGF-β binding partners [18–23].

This review focuses on the expression of DSPP-derived proteins and protein-protein interactions of these with TGF-β in porcine pulp and odontoblasts at both the protein and mRNA level.

2. DSPP-derived proteins in dentin and pulp

DSP is a proteoglycan containing the chondroitin-4-sulfate and chondroitin-6-sulfate chain. It forms covalent dimers and is detected as a smear band extending from 100–280 kDa on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) [24]. Previously, we isolated full-length DSP, various N-terminal fragments of DSP, the DSP proteoglycan core, and extended DGPs [4]. DGP is a phosphorylated glycoprotein having an apparent molecular weight of 19 kDa. DPP is a highly phosphorylated protein having an isoelectric point near 1.1 [25]. Porcine DPP averages 155 phosphates per molecule and appears mainly as a doublet of bands migrating at 95 kDa on SDS-PAGE (Fig. 1A) [26]. Due to its extensive phosphorylation, DPP can be specifically detected by Stains-all staining on SDS-PAGE gels, and is not detected by standard Coomassie Brilliant Blue (CBB) staining. We recently isolated, using heparin affinity chromatography, DSPP-derived proteins from pulp tip (PT) or pulp body (PB) extracts [27] (Fig. 1B). DSPP-derived proteins migrated as a smear band extending from 100–280 kDa on SDS-PAGE (Fig. 1C) and western blots (not shown).

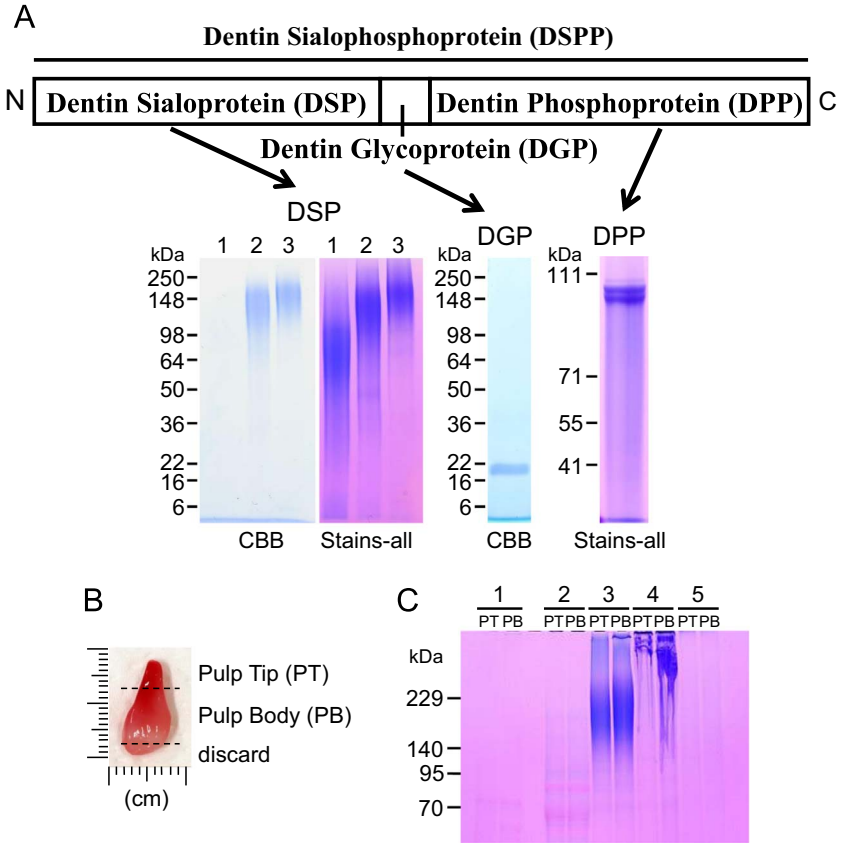

Fig. 1. DSPP-derived proteins in porcine incisor pulp and dentin. (A) Scheme for the location of DSPP and DSPP-derived proteins using SDS-PAGE (5–20% gradient gel for DSP and DGP, and 3–8% gradient gel for DPP) stained with Simply Blue (CBB) or Stains-all. The gels on the left show three DSPs: DSP core (lane 1), DSP (lane 2) and DSP-DGP complex (lane 3); middle gel, DGP; and gel on right, DPP in dentin. (B) Permanent incisor pulp from 5-month-old pig. The pulp tip (PT), pulp body (PB) and bottom part were excised with a razor blade [27]. (C) SDS-PAGE (5–20% gradient gel) stained with Stains-all showing five fractions (1–5) isolated by heparin sepharose chromatography [27].

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