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Review Article

Structural features, processing mechanism and gene splice variants of dentin sialophosphoprotein

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Summary Dentin sialophosphoprotein (DSPP) plays an important role in the formation of dentin. Understanding its structure and function would provide important insights into the regulation of dentin mineralization. For the past 15 years, we have been studying DSPP-derived proteins isolated from pig dentin. Porcine DSPP is synthesized and secreted by odontoblasts and processed into three proteins, i.e., dentin sialoprotein (DSP), dentin glycoprotein (DGP), and dentin phosphoprotein (DPP), by bone morphogenetic protein 1 and matrix metalloproteinase-20 and -2. DSP is a proteoglycan that forms covalent dimers, DGP is a phosphorylated glycoprotein, and DPP is a highly phosphorylated intrinsically disordered protein with genetic polymorphisms. Furthermore, DPP is not detected in dental pulp. This is possibly due to the existence of two mRNA variants of the *DSPP* gene: one that encodes the DSP region alone and another that encodes full-length DSPP. The mRNA variant encoding DSP alone is expressed in dental pulp and odontoblasts, but the variant encoding full-length DSPP is predominantly expressed in odontoblasts and barely in dental pulp.

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1. Introduction

Dentin is the hard tissue that constitutes the body of a tooth and is involved in the protection of the dental pulp within and the support of the overlying enamel and cementum. On a weight basis, dentin consists of approximately 70% inorganic substances, 20% organic substances, and 10% water. Of the organic substances, approximately 90% is type I collagen [1] and the remaining 10% comprises non-collagenous proteins. The largest component of the non-collagenous proteins is the protease-processed products of dentin sialophosphoprotein (DSPP) [2]. Porcine DSPP comprises three proteins, namely, dentin sialoprotein (DSP), dentin glycoprotein (DGP), and dentin phosphoprotein (DPP) [3–6]. Genetic studies have shown that type I collagen and DSPP are critical for the formation of human dentin. Presently, hereditary dentin defects are classified as dentin dysplasia (types I and II) and dentinogenesis imperfecta (types I–III) [7]. Mutation of the *DSPP* gene (4q21) is especially known to cause dentin dysplasia type II and dentinogenesis imperfecta types II and III [8–15], making the pursuit of studies on DSPP at both the genetic and protein levels very meaningful. However, most protein-level studies of DSPP have been performed with rodent teeth thus far, leaving many issues unresolved, such as (1) the expression levels of DSP and DPP in dentin are different (DSP: 5–8%, DPP: 60%), even though they are both synthesized from the same *DSPP* gene; (2) DGP has not been identified in rodent teeth, as opposed to DPP; (3) polymorphisms of DPP have not been characterized; (4) the processing mechanism of DSPP has not been elucidated; (5) the function of DSP has not been explained; and (6) no information is available on the linker regions. Therefore, we decided to perform protein-level studies of DSPP using second molars from approximately 5-month-old pigs as an experimental model (Figure 1), as they: (1) yield larger quantities of each DSPP-derived protein than rodents; (2) display high homology to the human *DSPP* gene; and (3) can be obtained cheaply. In this review, our observations based on our results so far on the structures and processing mechanisms of DSPP-derived proteins and their gene expression will be presented, as well as their possible functions and usefulness in future studies.

2. DSPP-derived proteins

2.1. Dentin sialoprotein (DSP)

DSP was discovered approximately 35 years ago as the most prominent electrophoretic band among the rat dentin non-collagenous proteins [16]. Its molecular weight was approximately 95 kDa on electrophoresis, but was measured as 52.57 kDa with a sedimentation equilibrium method, containing approximately 29.6% carbohydrates [17]. On the basis of the N-terminal amino acid sequence of rat DSP (IPVPQLV), cDNA cloning of DSP and DPP was performed. It was then reported that the translation of DSP terminated at the 366th amino acid [18], and the DSP and DPP domains were proposed to be of a so-called ''bicistronic nature,'' where one gene encodes two different proteins [19–21]. However, it was later proven by cloning mouse DSPP cDNA that DSP and DPP are in fact expressed from a single open reading frame, revealing DSP and DPP to be chimeric proteins whose genes form a single entity and are transcribed and expressed together as a single protein [2]. Studies on rat DSP protein were advanced further after this discovery, revealing DSP and DPP to be DSPP-derived proteins that are synthesized from the *DSPP* gene expressed in odontoblasts, secreted, and then subjected to protease processing [22]. However, many issues remained unresolved, as mentioned in the Introduction. My research group has launched our own DSPP protein investigations using pigs as our experimental model. As there was no information available on porcine DSPP genomic and cDNA sequences when we started, we constructed a unidirectional cDNA library derived from the pulp organ of developing pig teeth and screened for the *DSPP* gene. As a result, we succeeded in isolating a cDNA clone that only encodes DSP [23]. Using a glutathione-*S*-transferase (GST) fusion protein expression vector in *Escherichia coli*, we produced a recombinant DSP protein (GST-DSP) based on the isolated cDNA sequence, followed by the generation of an anti- DSP polyclonal antibody. In order to detect DSP in western blots with this antibody, we prepared pulverized dentin samples (40 g) from second molars of 5-month-old pigs. Proteins were extracted from these samples by sequential extraction with a tris-guanidine buffer (G1), an acetic acid solution (A), and an acetic acid-sodium

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