

Y. Yamakoshi^{1*}, S. Kinoshita²,
L. Izuhara³, T. Karakida¹, M. Fukae¹,
and S. Oida¹

¹Department of Biochemistry and Molecular Biology, School of Dental Medicine, Tsurumi University, 2-1-3 Tsurumi, Tsurumi-ku, Yokohama 230-8501, Japan; ²Department of Pediatric Dentistry, School of Dental Medicine, Tsurumi University, 2-1-3 Tsurumi, Tsurumi-ku, Yokohama 230-8501, Japan; and ³Division of Regenerative Medicine, The Jikei University School of Medicine, 3-25-8 Nishishinbashi, Minato-ku, Tokyo 105-0003, Japan; *corresponding author, yamakoshi-y@tsurumi-u.ac.jp

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ABSTRACT

Porcine dentin sialophosphoprotein (DSPP) is the most abundant non-collagenous protein in dentin. It is processed by proteases into 3 independent proteins: dentin sialoprotein (DSP), dentin glycoprotein (DGP), and dentin phosphoprotein (DPP). We fractionated DPP and DSP along with TGF- β activity by ion exchange (IE) chromatography from developing pig molars and measured their alkaline phosphatase (ALP)-stimulating activity in human periodontal (HPDL) cells with or without TGF- β receptor inhibitor. We then purified TGF- β -unbound or -bound DPP and DSP by reverse-phase high-performance liquid chromatography (RP-HPLC) using the ALP-HPDL system. The TGF- β isoform bound to DPP and DSP was identified as being TGF- β 1 by both ELISA and LC-MS/MS analysis. We incubated carrier-free human recombinant TGF- β 1 (CF-hTGF- β 1) with TGF- β -unbound DPP or DSP and characterized the binding on IE-HPLC using the ALP-HPDL system. When only CF-hTGF- β 1 was incubated, approximately 3.6% of the ALP-stimulating activity remained. DPP and DSP rescued the loss of TGF- β 1 activity. Approximately 19% and 10% of the ALP stimulating activities were retained by the binding of TGF- β to DPP and DSP, respectively. The type I collagen infrequently bound to CF-hTGF- β 1. We conclude that both DPP and DSP help retain TGF- β 1 activity in porcine dentin.

KEY WORDS: tooth, HPLC, cell culture, extracellular matrix (ECM), isolation and purification, phosphophoryn.

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DPP and DSP are Necessary for Maintaining TGF- β 1 Activity in Dentin

INTRODUCTION

Dentin is the predominant mineralized tissue comprising the body of a tooth. Collagen constitutes approximately 90% of the dentin organic matrix (Linde *et al.*, 1989). The non-collagenous proteins in dentin are dominated by DSPP-derived proteins, which are generated by proteolysis of dentin sialophosphoprotein (DSPP) (MacDougall *et al.*, 1997). Porcine DSPP is expressed and secreted by odontoblasts and is processed by BMP-1, MMP-20, and MMP-2 into 3 main components: dentin sialoprotein (DSP) (Butler *et al.*, 1992; Ritchie *et al.*, 1994), dentin glycoprotein (DGP) (Yamakoshi *et al.*, 2005b), and dentin phosphoprotein (DPP) (Linde *et al.*, 1980; Ritchie and Wang, 1996). Genetic studies have shown that *DSPP* mutations have been found in dentin dysplasia (DD) or dentinogenesis imperfecta (DGI) patients. DSPP is a multidomain protein with hundreds of post-translational modifications (Qin *et al.*, 2004). Porcine DSP forms covalent dimers and is a highly glycosylated proteoglycan containing both chondroitin-4- and -6-sulfate chains (Yamakoshi *et al.*, 2005a, 2011). The DGP has so far been characterized in pig and has a single N-glycosylation and 4 phosphoserines that are in a highly conserved region near its carboxyl-terminus (Yamakoshi *et al.*, 2005b). DPP is the highly phosphorylated carboxyl-terminal domain of DSPP. The coding region of DPP varies in length among different individual pigs without affecting function (Yamakoshi *et al.*, 2008). Expression of a DSP transgene in the *Dspp* null background partially recovered the null phenotype and showed that there are distinct roles for DSP and DPP in dentin mineralization, with DSP regulating the initiation of dentin mineralization, and DPP the maturation of dentin (Suzuki *et al.*, 2009). Recently, it was demonstrated that mouse carboxy-terminal DSP enhanced the attachment, migration, proliferation, and differentiation of human periodontal ligament stem cells regulating gene expression of tooth and bone markers, growth factors, and transcription factors (Özer *et al.*, 2013).

Besides DSPP-derived proteins, the bioactive component has been found in the extracellular matrix of dentin (Bègue-Kirn *et al.*, 1992). This inductive activity was inhibited by the neutralizing transforming growth factor- β (TGF- β) antibody (Bègue-Kirn *et al.*, 1992). The TGF- β isoforms have been extracted from the dentin matrices of both rabbit and human teeth (Cassidy *et al.*, 1997); TGF- β 1 is a predominant isoform, and approximately half of that is present in the active form (Smith *et al.*, 1998). The dentin matrix sequesters TGF- β and acts as a reservoir for its activity. Decorin and biglycan have already been proposed as potential binding partners (Hildebrand *et al.*, 1994;

Iozzo and Murdoch, 1996; Cam *et al.*, 1997; Schonherr *et al.*, 1998; Markmann *et al.*, 2000; Baker *et al.*, 2009).

Our purpose in this study was to isolate TGF- β -bound DPP and DSP, identify the bound TGF- β isoform, and determine the binding ability of DPP and DSP to TGF- β .

MATERIALS & METHODS

All experimental procedures involving the use of animals were reviewed and approved by the Ethics Committee of the Institute of Tsurumi University, Yokohama, Japan.

Preparation of Porcine Dentin

Tooth germs of permanent molars were surgically extracted from the mandibles of five-month-old pigs obtained from the Meat Market of Metropolitan Central Wholesale Market (Tokyo, Japan). The enamel organ epithelia (EOE) and dental pulp were removed by means of tissue forceps. The remaining hard tissue on molars was reduced to “dentin powder” by means of a jaw crusher (Retsch Inc., Newtown, PA, USA).

Sequential Extraction of Proteins from Dentin Powder of Molars

The sequential extraction of proteins from dentin powder (40 g) of molars was carried out by the use of our previous demineralization method with 0.17 HCl and 0.95% formic acid (Tsuchiya *et al.*, 2011). The supernatant obtained from 0.5 M acetic acid/2 M NaCl solution (AN extract) was used to isolate DSP and DPP.

Isolation of DPP and DSP from Molar Dentin

The AN extract (150 mg) was fractionated by IE chromatography in a Q-Sepharose Fast Flow column (1.6 x 20 cm; GE Healthcare UK Ltd, Little Chalfont, UK) by our previous method (Tsuchiya *et al.*, 2011). The DPP and DSP in the third (ANQ3) and high-molecular-weight (HMW)-DSP in the fourth (ANQ4) fractions were dialyzed against water, lyophilized, and characterized by SDS-PAGE and ALP-HPDL system with or without the TGF- β specific inhibitor, SB431542.

Isolation of Bioactive Fractions in Porcine DPP and DSP

Both ANQ3 and ANQ4 samples (5 mg) and carrier-free recombinant human TGF- β 1 (CF-hTGF- β 1) (Cell Signaling Technology, Danvers, MA, USA) (1 μ g) were fractionated by reverse-phase high-performance liquid chromatography (RP-HPLC) by our previous method (Yamakoshi *et al.*, 2006). Protein fractions were collected at every 3 mL. Each fraction was lyophilized and characterized by the ALP-HPDL system.

Identification and Quantitative Analysis of TGF- β 1 by Enzyme-linked Immunosorbent Assay (ELISA)

The bioactive fractions of ANQ3 and ANQ4 that enhanced ALP-inducing activity in HPDL cells were combined and lyophilized. Each lyophilized sample was dissolved in 200 μ L of phosphate

buffered saline (PBS), and aliquots (40 μ L each) were used for ELISA. The identification and quantitative analysis of TGF- β 1 was determined by a sandwich enzyme immunoassay method with a Quantikine ELISA kit (R&D Systems, Inc., Minneapolis, MN, USA).

Mass Spectrometry (LC-MS/MS)

LC-MS/MS was performed by Japan Bio Services Co., LTD (Asaka, Saitama, Japan) on the 13-kDa protein bands in ANQ3 and ANQ4 samples. Samples were analyzed by nano-LC/MS/MS on a ThermoFisher LTQ Orbitrap XL (Thermo Fisher Scientific Inc., Waltham, MA, USA).

SDS-PAGE and Western Blots

SDS-PAGE was performed with Novex 4% to 20% Tris-glycine or NuPAGE 4% to 12% Bis-Tris gels (Life Technologies/Invitrogen, Carlsbad, CA, USA). The gels were stained with Simply Blue Safe Stain (Life Technologies/Invitrogen) or Stains-All (Sigma-Aldrich). The apparent molecular weights of the protein bands were estimated by comparison with SeeBlue Plus2 Pre-Stained Standard (Life Technologies/Invitrogen). Duplicate gels were transblotted onto Invitrolon PVDF membrane (Life Technologies/Invitrogen) and immunostained with porcine N-terminal DSP and DGP polyclonal antibodies.

In vitro Binding Experiments

TGF- β 1-unbound DPP and DSP and neutral soluble type-I collagen (Nitta Gelatin, Osaka, Japan) (1 mg each) were incubated with 1 μ g of CF-hTGF- β 1 in 50 mM Tris-HCl buffer (pH 7.4) for 20 hr at 37°C. Each sample was fractionated by IE-HPLC in an Inertsil AX column (0.46 x 25 cm; GL Sciences Inc., Tokyo, Japan) run at a flow rate of 0.5 mL/min and monitored at 280 nm [buffer A, 50 mM Tris-HCl/6 M urea (pH 7.4); buffer B, 1 M NaCl/buffer A]. Proteins were eluted with a linear gradient of buffer B for 55 min at the flow rate of 0.5 mL/min, and 2-mL fractions were collected. Each fraction was de-salted and buffer-changed to 50 mM Tris-HCl buffer (pH 7.4) in an Amicon Ultra-3K (Merck KGaA, Darmstadt, Germany). Each fraction was concentrated to 20- μ L volume, and aliquots (5 μ L) were used for the ALP-HPDL system. TGF- β 1-unbound DPP and DSP, neutral type-I collagen, and CF-hTGF- β 1 only were incubated and fractionated by IE-HPLC as controls.

Enzyme Assay (ALP-HPDL System)

Human periodontal ligament fibroblasts (HPDL) were purchased from LONZA (LONZA, Walkersville, MD, USA). The cell culture and ALP activity were performed according to our previous method (Nagano *et al.*, 2006). Positive controls included the use of recombinant hTGF- β 1 with carrier (0.3 ng/mL) (R&D Systems). The TGF- β 1 receptor inhibitor, SB431542, was applied to a final concentration of 1 mM into the ALP-HPDL system for examination of the influence against the ALP-inducing activity increased by the application of samples. In controls, the ALP-inducing activity in HPDL cells was enhanced by TGF- β 1.

RESULTS

DPP from Dentin of Molars

Since TGF- β has been found in rabbit and human incisor dentin (Cassidy *et al.*, 1997), we first decided to use porcine incisor dentin for our preliminary study and found DPP containing TGF- β activity (Appendix Figs. 1-3). Next we used porcine molars to obtain DPP containing TGF- β activity in quantity from developing pig teeth and confirmed that DPP in porcine molars behaves the same as DPP isolated from the incisor. We used IE chromatography to separate the AN extract into 4 fractions (Fig. 1A). The DPP eluted in the third fraction (ANQ3) and migrated as triplet bands at 98 kDa on SDS-PAGE, while the high-molecular-weight DSP eluted in the fourth fraction (ANQ4) and migrated as a smear band at approximately 100 to 250 kDa on SDS-PAGE (Fig. 1B). Immunopositive bands at approximately 150 and 250 kDa, with the N-DSP antibody, were also identified in the ANQ3 fraction, while both N-DSP and DGP antibodies strongly recognized protein bands at over 250 kDa (Fig. 1C). Both ANQ3 and ANQ4 enhanced ALP-inducing activity in HPDL cells, and the SB431542 significantly decreased its activity (Fig. 1D).

Identification of TGF- β 1 in ANQ3 and ANQ4 Fractions

We further separated ANQ3 and ANQ4 samples into 23 fractions by RP-HPLC using a C18 column. The DSPP-derived proteins in ANQ3 and ANQ4 samples eluted within fractions 10 to 16 (Fig. 2A, top and middle), but the ALP-inducing activity in HPDL cells was in fractions 17 to 20 in both the ANQ3 and ANQ4 samples (Fig. 2B, top and middle). No ALP-inducing activity was detected when we ran CF-hTGF- β 1 only under this isolation system (Figs. 2A and 2B, bottom). Both SDS-PAGE and Western blot analyses showed that the trace amount of DPP was detected in fraction 17 in ANQ3 (Fig. 2C), and DSP was identified as two bands within fractions 17 to 20 (Fig. 2D). We interpret these findings to indicate that an *in vivo* bioactive molecule, such as TGF- β in porcine dentin, binds to DPP and to DSP.

To learn more about the TGF- β in tooth dentin, we performed ELISA. An aliquot of fractions 17 to 20 in ANQ3 and in ANQ4 was assayed by ELISA. Each sample in ANQ3 and ANQ4 was positive against 2 TGF- β 1 antibodies and contained approximately 270 and 380 pg of TGF- β 1 *per* mg of ANQ3 and ANQ4, respectively (Fig. 2E). We furthermore attempted to characterize TGF- β 1 by LC-MS/MS analysis. The LC-MS/MS analysis gave a part of the TGF- β 1 protein sequence corresponding to Q²⁹⁷-K³¹⁵ (Appendix Fig. 4).

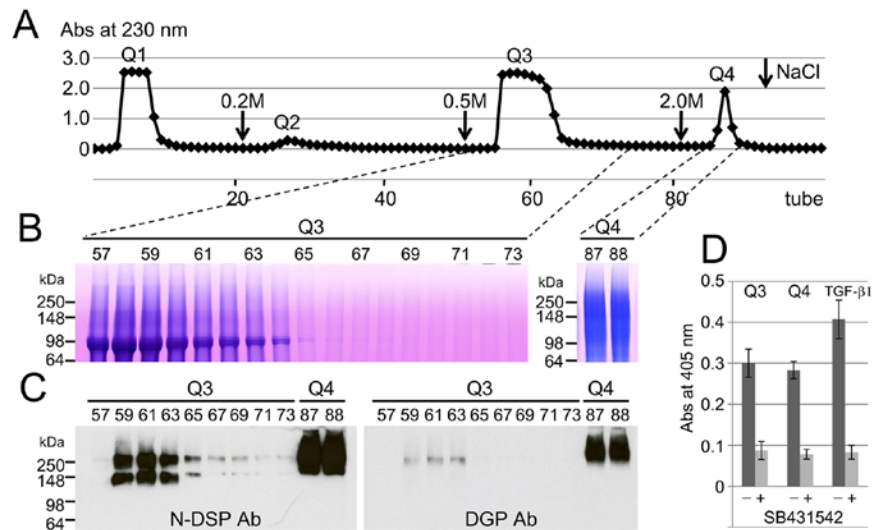


Figure 1. Isolation of DSPP-derived proteins in porcine molar dentin. **(A)** Q-Sepharose chromatograms showing absorbance at 230 nm for AN extracts from dentin of porcine developing molars (150 mg). Downward-pointing arrows are the starting point of the step gradient with 0.2, 0.5, and 2 M NaCl. **(B)** SDS-PAGE (4% to 20% gradient gel) stained with Stains-All showing each tube on a Q-Sepharose chromatogram. **(C)** Western blots showing each tube on a Q-Sepharose chromatogram with specific antibodies against N-terminal dentin sialoprotein (N-DSP Ab, left) and dentin glycoprotein (DGP Ab, right), showing isolated DSP in Q3 and Q4 fractions. **(D)** ALP-inducing activity of HPDL cells exposed by Q3 and Q4 fractions and TGF- β 1 (0.3 ng/ml) without (-) or with (+) SB431542. Data are means \pm SE of 3 culture wells.

In vitro Binding Experiments

To gain more information about the binding potential of DPP and DSP to TGF- β 1, we performed *in vitro* binding experiments. For this study, we used TGF- β 1-unbound DPP and DSP obtained from fractions 10 to 12 in ANQ3 and 12 to 15 in ANQ4, respectively. Since the CF-hTGF- β 1 lost its activity only during RP-HPLC (Fig. 2B, bottom), we changed the isolation system from RP-HPLC to IE-HPLC. The CF-hTGF- β 1 eluted into fractions 6 to 8 by IE-HPLC, which enhanced ALP-inducing activity after the incubation with HPDL cells (Figs. 3A, 3B). The TGF- β 1-unbound DSP eluted into fractions 10 to 12 and enhanced ALP-inducing activity after CF-hTGF- β 1 binding (Figs. 3C, 3D). The DSP protein band on SDS-PAGE correlated with positions of ALP-inducing activity (Fig. 3E). The TGF- β 1-unbound DPP eluted into fractions 8 to 11 by IE-HPLC. Those fractions enhanced ALP-inducing activity after CF-hTGF- β 1 binding (Figs. 3F, 3G). The DPP band migrated at the same positions with ALP-inducing activity (Fig. 3H). We also tested the binding potential of the neutral soluble type I collagen against the CF-hTGF- β 1. The type I collagen eluted into 9 to 12 by IE-HPLC (Fig. 3I). The CF-hTGF- β 1 was also able to bind to type I collagen in fractions 9 to 12, and those fractions weakly enhanced ALP-inducing activity (Fig. 3J). The ALP-inducing activity from unbound CF-hTGF- β 1 isolated by IE-HPLC was found in fractions 6 to 8, with no detectable collagen bands on SDS-PAGE (Fig. 3K). Total amounts (ng) of unbound or bound TGF- β 1 in fractions obtained from IE-HPLC after binding experiments against one mg of DSP, DPP, and type I collagen were calculated from the standard CF-hTGF- β 1 (0.3 ng/mL)

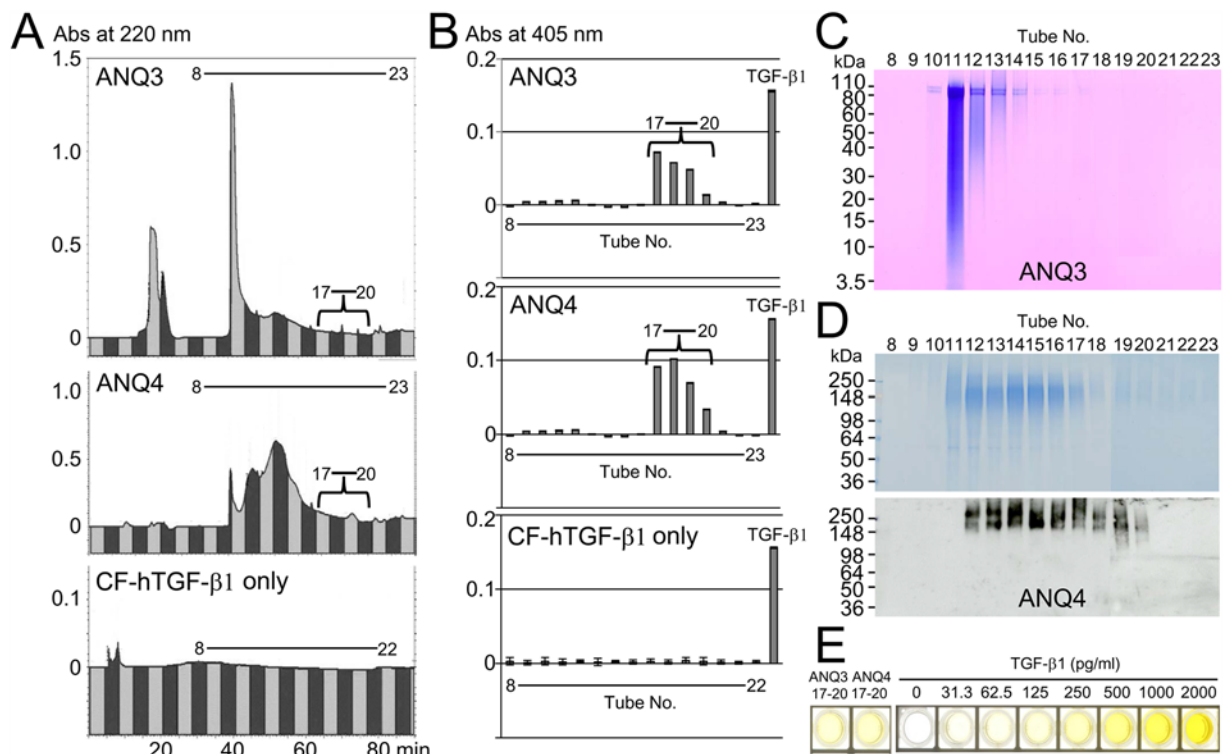


Figure 2. Isolation of TGF- β 1-unbound and -bound DPP and DSP in porcine molar dentin. **(A)** RP-HPLC chromatograms showing absorbance at 220 nm for ANQ3 and ANQ4 (5 mg each) fractionated by IE chromatography and for CF-hTGF- β 1 (1 μ g). **(B)** ALP-inducing activity of HPDL cells exposed by fractions 8-23 in ANQ3, ANQ4, and CF-hTGF- β 1. TGF- β 1 (0.3 ng/mL) is used as a positive control. Data are means \pm SE of 3 culture wells. **(C)** SDS-PAGE (4% to 12% gradient gel) stained with Stains-All showing each tube in ANQ3 fractionated by RP-HPLC. **(D)** SDS-PAGE (4% to 12% gradient gel) stained with Simply Blue (top) and Western blots (bottom) used specific antibodies against N-terminal dentin sialoprotein, showing each tube in ANQ4 fractionated by RP-HPLC. **(E)** ELISA for the detection of TGF- β 1 in combined fractions 17-20 in ANQ3 and ANQ4 enhanced ALP-inducing activity in HPDL cells. This figure is available in color online at <http://jdr.sagepub.com>.

and are shown in the Table. The CF-hTGF- β 1 only as control retained its activity during IE-HPLC, but its amount after the dilution correction was 36.4 ± 3.01 ng. Both DPP and DSP were able to bind CF-hTGF- β 1, and amounts of TGF- β 1 bound to DSP or DPP after the dilution correction were 101.8 ± 2.90 and 190.5 ± 7.34 ng/mg of DSP or DPP, respectively. The type I collagen was able to bind CF-hTGF- β 1 weakly, and its amount of TGF- β 1 bound to type I collagen after the dilution correction was 23.8 ± 2.04 ng/mg of type I collagen. The unbound CF-hTGF- β 1 against type I collagen was also found during IE-HPLC, and its amount was 24.7 ± 2.87 ng.

DISCUSSION

DPP from Dentin of Molars

DSPP-derived proteins are the most abundant non-collagenous proteins in porcine molar dentin (Yamakoshi *et al.*, 2011). Both DPP and DSP are expressed in stoichiometric amounts as the N-terminal and C-terminal domains of DSPP (MacDougall *et al.*, 1997) and are found in equal molar amounts in dentin (Yamakoshi *et al.*, 2008; Zhu *et al.*, 2010). Previously, we showed that porcine DPP and DSP can be found in the acid-NaCl or “AN” extract (Yamakoshi *et al.*, 2006, 2008; Tsuchiya *et al.*, 2011). We demonstrated that TGF- β co-eluted with DPP

and DSP into the ANQ3 fraction, and with HMW-DSP (*i.e.*, DSP-DGP complex) in ANQ4. The ALP-inducing activity in ANQ3 and ANQ4 fractions obtained from the IE chromatography under denatured conditions is due to the TGF- β bound to both DPP and DSP in porcine molar dentin.

Identification of TGF- β 1 in ANQ3 and ANQ4 Fractions

Using the porcine molar, which provides abundant dentin proteins, and having previously surveyed dentin extracts for the presence of DPP and DSP, we were able to quantify levels of TGF- β from porcine molars. Previously, we showed that DPP and DSP were well-separated by RP-HPLC with a C18 column (Yamakoshi *et al.*, 2006). Our study demonstrated that the ALP-inducing activity in HPDL cells was retained under the acidic condition of 0.05% TFA and 80% acetonitrile (pH \sim 2) during RP-HPLC and was detected in fractions 17 to 20 with minor amounts of DPP and DSP in ANQ3 and ANQ4 fractions, respectively. We first tried to identify TGF- β by using the CF-hTGF- β 1 as standard and by evaluating the same elution time on RP-HPLC. However, the CF-hTGF- β 1 alone lost its activity during RP-HPLC. This suggested that both DPP and DSP may function to preserve TGF- β activity. We determined that the approximate percentages of TGF- β -bound DPP and DSP *in vivo* are 1.5 and 2.6, from the calculation of the total area of

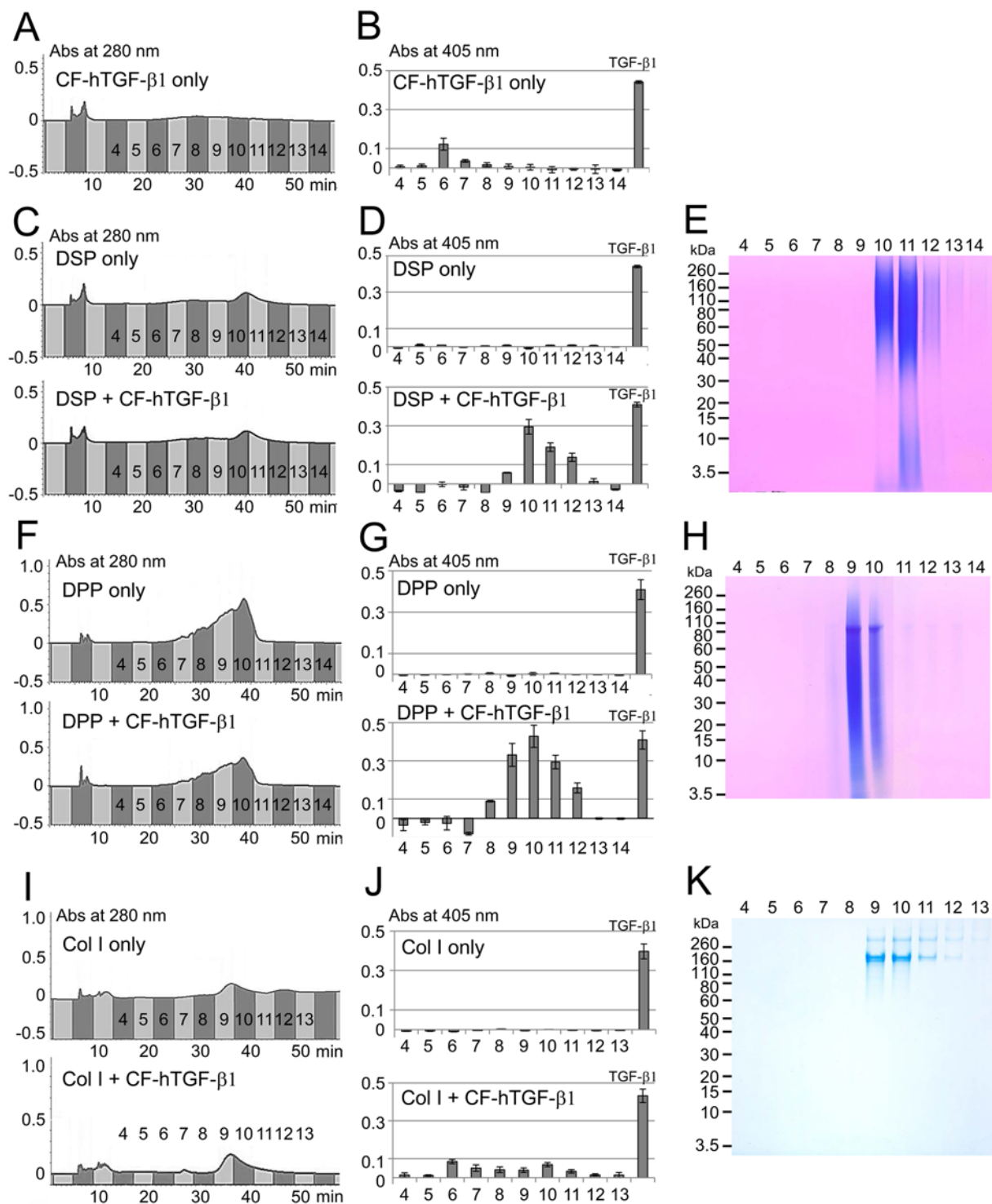


Figure 3. *In vitro* binding study of CF-hTGF- β 1 for DSP, DPP, and type I collagen. **(A, C, F, I)** IE-HPLC chromatograms showing absorbance at 280 nm for **(A)** CF-hTGF- β 1 only, **(C)** TGF- β 1-unbound-DSP only (DSP only, top) and TGF- β 1-bound DSP after binding experiment (DSP + CF-hTGF- β 1, bottom), **(F)** TGF- β 1-unbound-DPP only (top) and TGF- β 1-bound DPP after binding experiment (DPP + CF-hTGF- β 1, bottom), and **(I)** type I collagen only (Col I only, top) and TGF- β 1-bound collagen after binding experiment (Col I + CF-hTGF- β 1, bottom). **(B, D, G, J)** ALP-inducing activity of HPDL cells exposed by **(B)** fractions 4 to 14 in CF-hTGF- β 1 only, **(D)** TGF- β 1-unbound-DSP only (DSP only, top) and TGF- β 1-bound DSP (DSP + CF-hTGF- β 1, bottom), **(G)** TGF- β 1-unbound-DPP only (DPP only, top) and TGF- β 1-bound DPP (DPP + CF-hTGF- β 1), and **(J)** fractions 4 to 13 in type I collagen only (Col I only, top) and TGF- β 1-bound collagen (Col I + CF-hTGF- β 1, bottom). The recombinant human TGF- β 1 (TGF- β 1) (0.3 ng/mL) was used as positive control. Data are means \pm SE of 3 culture wells. **(E, H, K)** SDS-PAGE (4% to 12% gradient gel) stained with Stains-All showing **(E)** DSP in fractions 4 to 14, **(H)** DPP in fractions 4 to 14, and **(K)** stained with Simply Blue showing type I collagen in fractions 4 to 13 isolated by IE-HPLC after binding to CF-hTGF- β 1. This figure is available in color online at <http://jdr.sagepub.com>.

Table. Amount of Unbound or Bound TGF- β 1 for DSP, DPP, and Type I Collagen

| Binding Protein | (ng) | |
|-------------------------|-----------------|------------------|
| | Unbound | Bound |
| CF-hTGF- β 1 only | 36.4 \pm 3.01 | Not detected |
| DSP | Not detected | 101.8 \pm 2.90 |
| DPP | Not detected | 190.5 \pm 7.34 |
| Col I | 24.7 \pm 2.87 | 23.8 \pm 2.04 |

The value indicates total ng of TGF- β 1 obtained from IE-HPLC after binding experiments against 1 mg of protein.

TGF- β -unbound DPP and DSP (*i.e.*, fractions 10-16 in ANQ3 and ANQ4) and TGF- β -bound DPP and DSP (*i.e.*, fractions 17-20 in ANQ3 and ANQ4) on RP-HPLC.

The TGF- β family consists of 5 closely related isoforms, which are derived from structurally related genes (Massagué, 1990). TGF- β 1 is the only isoform detected in human dentin and the major isoform in rabbit dentin, where small amounts of TGF- β 2 and TGF- β 3 isoforms have been detected (Cassidy *et al.*, 1997). In this study, we were able to identify the TGF- β 1 isoform bound to DPP and DSP in ANQ3 and ANQ4 fractions. In addition, the concentrated mixture of fractions 17 to 20 was enough to identify a part of the amino acid sequence of TGF- β 1 by LC-MS/MS analysis.

In vitro Binding Experiments

We focused our *in vitro* binding study on the type I collagen- and DSPP-derived proteins because of the major protein and most abundant non-collagenous protein in dentin (Yamakoshi *et al.*, 2011). It has been shown that the collagen alone does not interact with TGF- β (Schonherr *et al.*, 1998). We demonstrated that the neutral type I collagen was not easy to bind to CF-hTGF- β 1. When only 1 μ g of CF-hTGF- β 1 was incubated and run on IE-HPLC, approximately 3.6% of activity remained. DPP and DSP were able to rescue the loss of TGF- β 1 activity, and approximately 19% and 10% of its activity were retained by binding to DPP and DSP, respectively. Thus, our *in vitro* study demonstrated that both DPP and DSP are necessary for maintaining TGF- β 1 activity.

Other *in vitro* studies have demonstrated that both decorin and biglycan are able to bind to TGF- β and are candidates for the sequestration of TGF- β reservoirs (Hildebrand *et al.*, 1994; Iozzo and Murdoch, 1996; Hyttiäinen *et al.*, 2004; Baker *et al.*, 2009). The decorin contains independent binding sites for TGF- β and type I collagen (Schonherr *et al.*, 1998). DSP is the most abundant proteoglycan in dentin and is therefore likely to interact with collagen. Conversely, DPP binding with collagen improves its structural stability (Yamakoshi *et al.*, 2008). Such DSP-collagen and DPP-collagen interactions may sterically hinder the binding of TGF- β isoforms *in vivo*. In this study, we demonstrated that both DPP and DSP provide a retention capability for bioactive molecules such as TGF- β . During dentinogenesis, the TGF- β 1 bound to DPP and DSP is liberated in association with the degradation of DPP and DSP and may facilitate reparative dentin formation by affecting the odontoblast. It also

may affect cell behavior in the dentin-pulp complex following tissue injury. Studies are now required to examine the interaction of DPP and DSP with TGF- β 1 and collagen, and the bio-availability of such a complex for the future.

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