Amelogenin Stimulates Bone Sialoprotein (BSP) Expression Through Fibroblast Growth Factor 2 Response Element and Transforming Growth Factor-β1 Activation Element in the Promoter of the BSP Gene

Emi Shimizu,*† Ryoichiro Saito,* Youhei Nakayama,* Yu Nakajima,* Naoko Kato,* Hideki Takai,* Dong-Soon Kim,* Masato Arai,* James Simmer,‡ and Yorimasa Ogata*†

Background: Amelogenins are a complex mixture of hydrophobic proteins that are the major organic component of developing enamel. The principal function of the amelogenins and their degradation products has been assigned to structural roles in creating the space and milieu for promoting enamel mineralization. Enamel matrix derivative (EMD) has been used clinically for periodontal regeneration and its therapeutic effectiveness has been attributed to amelogenin, non-amelogenin enamel matrix proteins, and growth factors. While EMD is believed to induce periodontal regeneration, the precise mechanism is not known. Bone sialoprotein (BSP), an early phenotypic marker of osteoblast and cementoblast differentiation, has been implicated in the nucleation of hydroxyapatite during bone formation. In this study, we examined the ability of amelogenin to regulate BSP gene transcription in osteoblast like cells.

Methods: We conducted Northern hybridization, transient transfection analyses, and gel mobility shift assays using full-length recombinant amelogenin to determine the molecular basis of the transcriptional regulation of BSP gene by amelogenin.

Results: Recombinant amelogenin (1 μg/ml, 12 hours) increased BSP mRNA levels ~2.4-fold. In transient transfection analyses, amelogenin (1 μg/ml, 12 hours) increased luciferase activity ~1.5-fold in pLUC3 (nucleotides –116 to +60) and further increased pLUC5 (nucleotides –801 to +60) activity ~2.3-fold transfected into ROS 17/2.8 cells. Amelogenin also increased luciferase activities in rat stromal bone marrow cells. The effect of amelogenin was inhibited by the tyrosine kinase inhibitor herbimycin A. Transcriptional stimulation by amelogenin was almost completely abrogated in cells expressing a BSP promoter construct with a mutation in the fibroblast growth factor 2 (FGF2) response element (FRE). Gel mobility shift assays with radiolabeled FRE and transforming growth factor-β1 (TGF-β1) activation element (TAE) ds-oligonucleotides revealed increased binding of nuclear proteins from amelogenin-stimulated ROS 17/2.8 cells.

Conclusion: Amelogenin stimulation alters BSP gene transcription by inducing nuclear proteins that bind to the FRE and TAE in the rat BSP gene promoter. *J Periodontol 2005;76:1482-1489.*

KEY WORDS

Amelogenin; animal studies; bone and bones; dental enamel; enamel matrix derivative; gene transcription; growth factors, FGF2 response element; growth factors, fibroblast; growth factors, transforming; sialoglycoproteins.

* Department of Periodontology, Nihon University School of Dentistry at Matsudo, Chiba, Japan.
† Research Institute of Oral Science, Nihon University School of Dentistry at Matsudo.
‡ Department of Biologic and Materials Sciences, University of Michigan School of Dentistry, Ann Arbor, MI.

Amelogenins constitute 90% of the extracellular matrix secreted by ameloblasts, and the proteins are cleaved in a regulated process during enamel maturation.[1,2] It has been suggested that these proline-rich proteins play a role in the mineralization and structural organization of developing enamel.[3] Amelogenin is a cell adhesion protein,[4] a potential regulator of cementum-associated genes such as bone sialoprotein (BSP) and type I collagen, and specific amelogenin gene splice products may be tissue-specific epithelial mesenchymal signaling molecules.[5] Alternative splicing of the primary transcript as a potential mechanism for generating amelogenin heterogeneity.[6] Amelogenin-deficient mice display an amelogenesis imperfecta phenotype, showed root resportion, and reduced expression of BSP.[7-9] Enamel matrix derivative (EMD), the acid extracts of porcine cheese-like enamel matrix, has been developed as a clinical treatment to promote periodontal regeneration.[10,11] Approximately 90% of the protein in the EMD is amelogenins, and the remaining 10% is comprised of non-amelogenin enamel matrix proteins and growth factors.[12-14]

BSP is a mineralized tissue-specific protein that is glycosylated, phosphorylated, and sulfated.[15,16] BSP nucleates hydroxyapatite crystal formation[17,18] and is expressed in several malignant cancers where it is associated with the formation of ectopic hydroxyapatite microcrystals.[19,20] To study the transcriptional regulation of BSP, rat, human, and mouse BSP gene promoters were cloned and characterized.[21-24] These promoters include an inverted TATA box[25] overlapping a vitamin D response element,[26] an inverted CCAAT box (–50 to –46), which is bound with NF-Y transcription factor.[27,28] A cAMP response element (CRE; –75 to –68),[29,30] a fibroblast growth factor 2 response element (FRE; –92 to –85),[30-32] a pituitary-specific transcription factor-1 (Pit-1) motif (–111 to –105) which mediates the stimulatory effects of parathyroid hormone,[32,33] and a homeodomain binding element (HOX; –199 to –192) have been characterized.[11,34] Further upstream in the rat promoter a transforming growth factor-β (TGF-β activation element (TAE; –499 to –485)[11,35] and a glucocorticoid response element (–920 to –906), overlapping an activator protein (AP)-1 site (–921 to –915) have also been identified.[16,36]

Although EMD is used clinically, little is known about amelogenin effects on bone metabolism. Therefore, we wish to determine the effect of amelogenin on BSP gene expression. The aim of the present study was to evaluate the ability of amelogenin to regulate osteoblast specific gene expression such as BSP. In this study we show that amelogenin regulates BSP expression in osteoblast-like ROS 17/2.8 cells through FRE and TAE in the rat BSP promoter.

MATERIALS AND METHODS

Cell Culture

The rat osteoblast-like ROS 17/2.8 cells[37] and rat stromal bone marrow cells (SBMC)[38] were maintained in alpha minimum essential medium (α-MEM)§ containing 10% fetal calf serum (FCS).§ Cells were grown to confluence in 60 mm tissue culture dishes and then cultured in α-MEM without serum and incubated with or without recombinant amelogenin for time periods extending from 3 to 12 hours. RNA was isolated from triplicate cultures at various time intervals and analyzed for the expression of BSP mRNA by Northern hybridization as described below.

Preparation of Recombinant Porcine Amelogenin

Recombinant porcine amelogenin (rP172) was expressed from the pET11 expression vector in *E. coli* BL21(DE3) cells‖ and purified from *E. coli* extracts by selective precipitation in ammonium sulfate (20% saturation), followed by ion exchange chromatography, followed by separation on a C4 reversed-phase column.[3]

Northern Hybridization

Aliquots (20 µg) of total RNA were fractionated on 1.2% agarose gel and transferred onto a hybond-N+ membrane.¶ Hybridizations were performed at 42°C with either a ^{32}P-labeled rat BSP, osteopontin (OPN), or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe. Following hybridization, membranes were washed four times for 5 minutes each at 21°C in 300 mM sodium chloride and 30 mM trisodium citrate pH 7.0 containing 0.1% sodium dodecyl sulfate. This was followed by two 20 minute washes at 55°C in 15 mM sodium chloride and 1.5 mM trisodium citrate pH 7.0, 0.1% SDS. The hybridized bands were scanned in an imaging analyzer# and normalized to the expression of GAPDH.[31]

Transient Transfection Assays

Exponentially growing cells were used for transfection assays. Twenty-four hours after plating, cells at 50% to 70% confluence were transfected using a lipofectamine reagent.** The transfection mixture included 1 µg of a luciferase (LUC) construct[16] and 2 µg pSV-ß-galactosidase (ß-gal) vector†† as an internal control to normalized for individual transfection efficiencies. Two days post-transfection, cells were deprived of serum for 12 hours and recombinant amelogenin (1 µg/ml) was added and the cells cultured for a further 12 hours prior to harvesting. The luciferase assay was performed according to the

§ GIBCO BRL Life Technologies, Tokyo, Japan.
‖ Stratagene, La Jolla, CA.
¶ Amersham Biosciences, Piscataway, NJ.
# Fuji BAS 2000, Fuji film, Tokyo, Japan.
** Invitrogen Corp., Carlsbad, CA.
†† Promega Corp., Madison, WI.

supplier's protocol‡‡ using a luminescence reader§§ to measure the luciferase activity. The protein kinase inhibitor H89 (5 μM) and H7 (5 μM) was used to inhibit protein kinase A and C. Herbimycin A (1 μM) was used for tyrosine kinase inhibition.[30,31] Oligonucleotide-directed mutagenesis by polymerase chain reaction (PCR) was utilized to introduce dinucleotide substitutions using a site-directed mutagenesis kit.‖ All constructs were sequenced as described previously to verify the fidelity of the mutagenesis.[31]

Gel Mobility Shift Assays

Confluent ROS 17/2.8 cells in T-75 flasks incubated for 3 and 12 hours with amelogenin (1 μg/ml) in α-MEM without serum were used to prepare nuclear extracts as described previously.[33] Double-stranded oligonucleotides encompassing the inverted CCAAT (nucleotides –61 to –37, 5′-CCGTGACCGTG**ATTGG**CTGCT GAGA), FGF2 response element (FRE; nucleotides –98 to –79, 5′-TTTTCT**GGTGAGAA**CCCACA), homeodomain binding element (HOX; nucleotides –204 to –179, 5′-TCCTCAGCCTT**CAATTAAA**TCCCACA) and TGF-β activation element (TAE; nucleotides –506 to –482, 5′-CAAAGCC**TTGGC**AGCCC**GGCTG**GCT) in the rat BSP promoter were prepared commercially,‖‖ while consensus NF1 (5′-CCTTTGGCATGCTGCCAATAT) were purchased.†† For gel shift analysis the double-stranded oligonucleotides were end-labeled with [γ-^{32}P] ATP and T4 polynucleotide kinase. Nuclear protein extracts (3 μg) were incubated for 20 minutes at room temperature (RT = 21°C) with 0.1 pM radiolabeled double-stranded-oligonucleotide in buffer containing 50 mM KCl, 0.5 mM EDTA, 10 mM Tris-HCl (pH 7.9), 1 mM DTT, 0.04% nonidet P-40, 5% glycerol, and 1 μg poly (dI-dC). Following incubation, the protein-DNA complexes were resolved by electrophoresis on 5% nondenaturing acrylamide gels (38:2 acrylamide/bis acrylamide) run at 150 V at RT. Following electrophoresis, the gels were dried and autoradiograms prepared and analyzed using an imaging analyzer.

Statistical Analysis

Triplicate samples were analyzed for each experiment and experiments replicated to ensure consistency of the responses to amelogenin. Significant differences between control and amelogenin treatment were determined using the Student *t* test.

RESULTS

Stimulation of BSP mRNA Expression in ROS 17/2.8 Cells

To study the regulation of BSP expression by amelogenin, we performed Northern hybridization analysis of total RNA extracted from osteoblastic ROS 17/2.8 cells. First, a dose-response relation for amelogenin induction of BSP was established by treating the ROS 17/2.8 cells with different concentrations of recombinant amelogenin for 12 hours. Amelogenin increased BSP mRNA levels at 0.01~1 μg/ml and had a maximal effect at 1 μg/ml (~2.4-fold) (Fig. 1A). Thus, 1 μg/ml amelogenin was used to determine the time course of BSP mRNA expression (Fig. 1B). Amelogenin upregulated BSP mRNA accumulation at 12 hours, whereas no effect on OPN and GAPDH mRNA was observed.

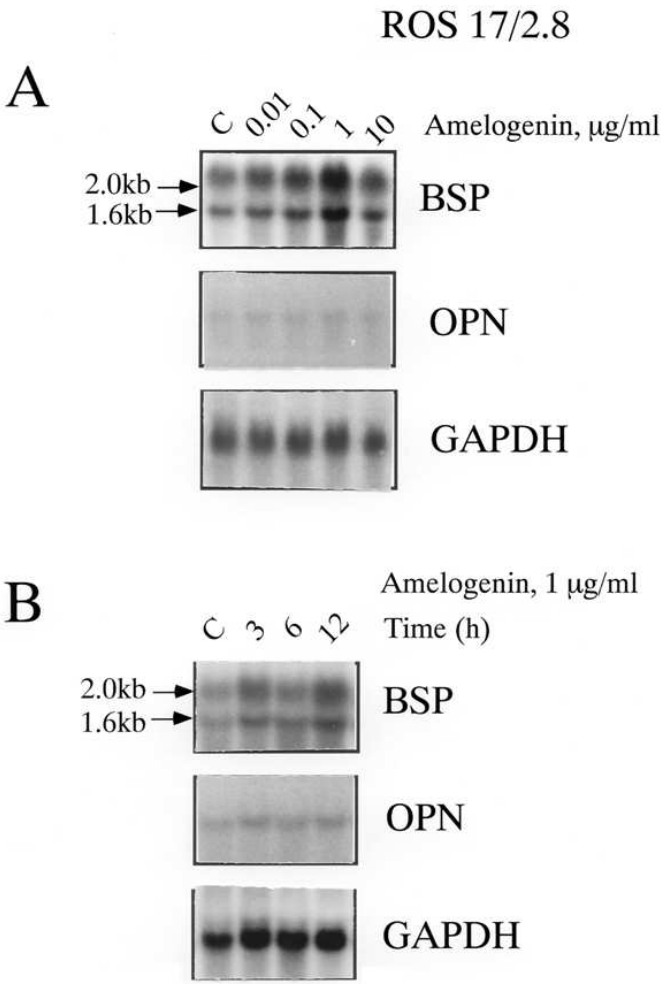

Figure 1.
Northern hybridization analysis of recombinant amelogenin effects on BSP mRNA expression. ***A)*** *Dose-response effect of amelogenin on BSPmRNA levels in the osteoblastic cell line ROS17/2.8 treated for 12 hours. At 0.01 to 10 μg/ml, amelogenin increased BSPmRNA with a maximal effect at 1 μg/ml.* ***B)*** *Twelve-hour time-course revealed an increase in BSP mRNA at 3 and 12 hours after the administration of 1 μg/ml amelogenin to ROS17/2.8 cells. Total RNA was isolated from triplicate cultures harvested after incubation times of 3, 6, and 12 hours and used for Northern hybridization analysis using a ^{32}P-labeled rat BSP DNA probe, an osteopontin DNA probe, and a GAPDH DNA probe.*

‡‡ PicaGene, Toyo Inki, Tokyo, Japan.
§§ Aloka, Tokyo, Japan.
‖‖ Bio-Synthesis, Inc., Lewisville, TX.

Transient Transfection Analysis of Rat BSP Promoter Constructs

Transient transfection of chimeric constructs, encompassing different regions of the rat BSP promoter driving a luciferase reporter gene (pLUC1~pLUC5), were performed in ROS 17/2.8 cells. The results of transfection assays (Fig. 2) indicated a ~1.5-fold (pLUC3; –116 to +60), ~1.5-fold (pLUC4; –425 to +60) and ~2.3-fold (pLUC5; –801 to +60) increase in transcription with the luciferase constructs after 12 hours of treatment with 1 µg/ml amelogenin. In shorter constructs (pLUC1; –18 to +60, pLUC2; –43 to +60), luciferase activities were not increased by amelogenin. When transcriptional activities in response to amelogenin were analyzed in normal rat SBMC, the transcriptional activities of pLUC3 (~1.5-fold), pLUC4 (~1.5-fold) and pLUC5 (~1.7-fold) were increased (Fig. 3). Included within the DNA sequence that is unique to pLUC3 construct (nucleotides –116 to –43) is an inverted CCAAT box (ATTGG; between nucleotides –50 and –46), a CRE (between nucleotides –75 and –68), FRE (between nucleotides –92 and –85), and a Pit-1 motif (between nucleotides –111 and –105) (Fig. 4). There are HOX (TCAATTAAAT, nucleotides –194 to –185) and TGF-β activation elements (TAE; C**TTGGC**AGCCC**GGCTG**G, nucleotides –500 to –484) in the pLUC4 and pLUC5 which were identified as enamel matrix derivative (EMD) response elements in the previous study (Fig. 4).[11] Since protein kinases mediate amelogenin signaling, we also investigated the effects of the protein kinase C inhibitor H7, the protein kinase A inhibitor H89, and the tyrosine kinase inhibitor HA on amelogenin-mediated transcription. Although amelogenin induction of pLUC3 promoter activity was inhibited by HA, no effects were observed for PKC and PKA (Fig. 5), indicating involvement of tyrosine kinase in the signaling pathway. Next we introduced mutations in the possible response elements encoded within nucleotides –116 to –43 of pLUC3 as shown in Figure 6. Whereas mutations in the CRE (M-CRE) and Pit-1 (M-PIT) had no effect on amelogenin stimulation,

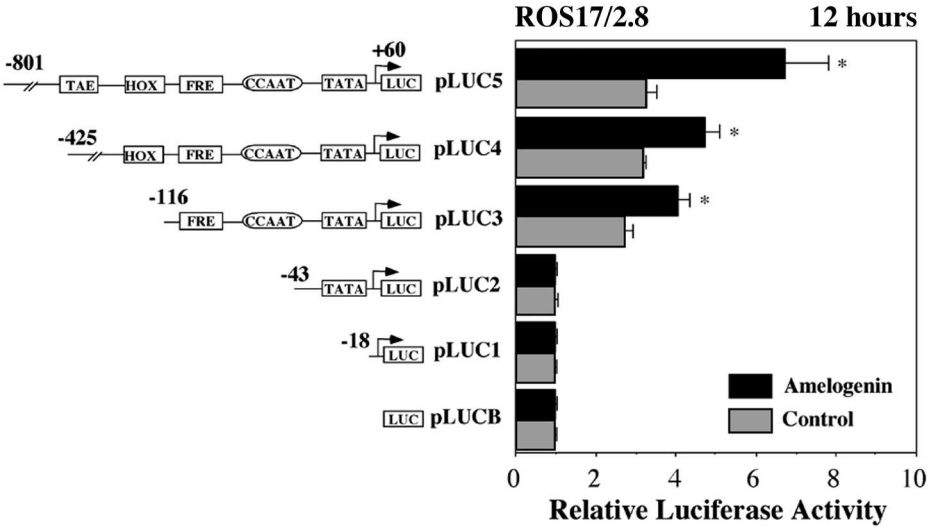

Figure 2.
*Amelogenin upregulates BSP promoter activity. Transient transfections of ROS 17/2.8 cells, in the presence or absence of amelogenin (1 µg/ml) for 12 hours, were used to determine transcriptional activity of chimeric constructs that included various regions of the BSP promoter ligated to a luciferase reporter gene. The results of transcriptional activity obtained from three separate transfections with constructs; pLUC basic (pLUCB) and pLUC1 to pLUC5 have been combined and the values expressed with standard errors. *Significant differences from control (relative luciferase activity of pLUCB), P <0.1.*

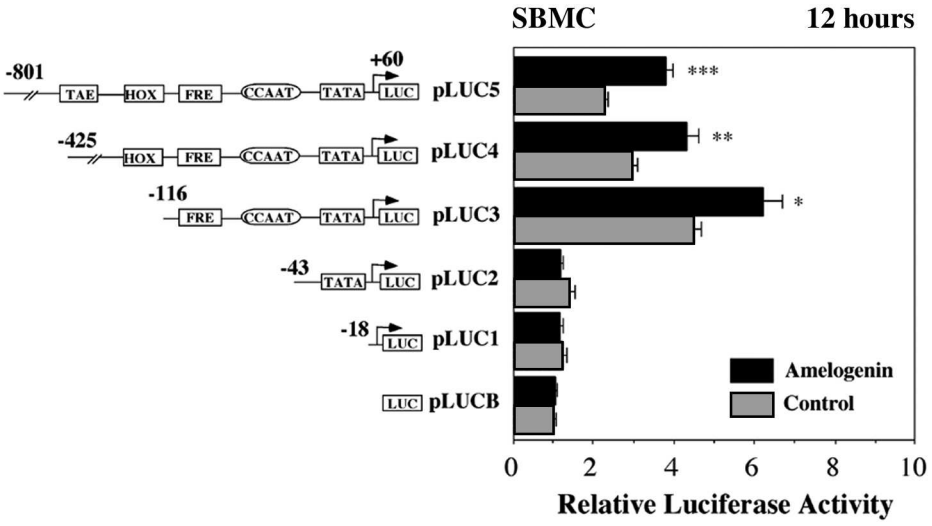

Figure 3.
*Transient transfections of rat stromal bone marrow cells. SBMC cells treated with or without amelogenin (1 µg/ml) for 12 hours were used to determine transcriptional activity of chimeric constructs that included various regions of the BSP promoter ligated to a luciferase reporter gene. The results of transcriptional activity obtained from three separate transfections with constructs; pLUC basic (pLUCB) and pLUC1 to pLUC5 have been combined and the values expressed with standard errors. Significant differences from control (relative luciferase activity of pLUCB): *P <0.1; †P <0.05; ‡P <0.02.*

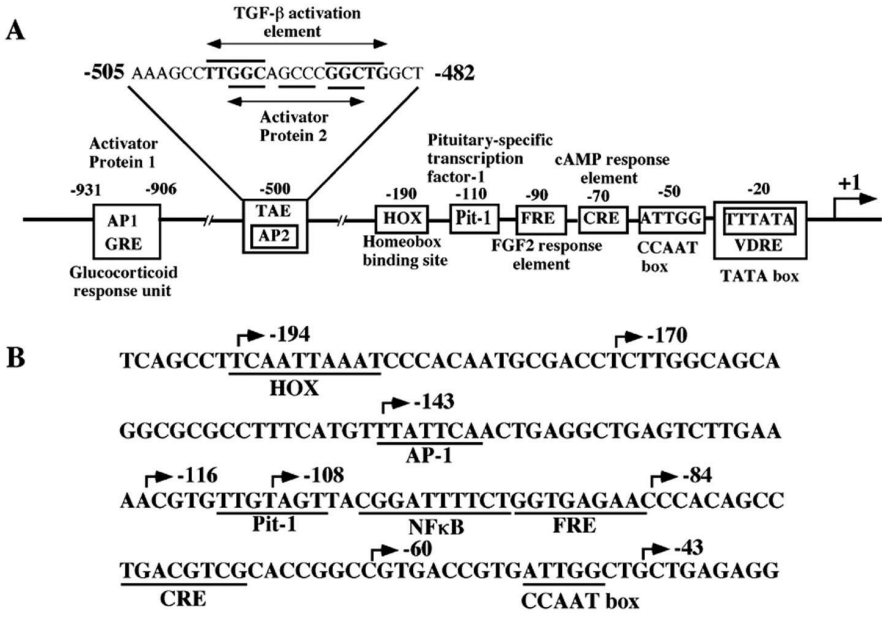

Figure 4.
Regulatory elements in the proximal rat BSP promoter. ***A)*** *The positions of the inverted TATA and CCAAT boxes, a cAMP response element (CRE), an FGF2 response element (FRE), a pituitary-specific transcription factor-1 (Pit-1), a homeobox-binding site (HOX), and a vitamin D response element (VDRE) that overlaps the inverted TATA box are shown in the proximal promoter region of the rat BSP gene, and a TGF-β1 activation element (TAE) overlapping an AP2 element and a glucocorticoid response elements (GRE) overlapping the AP1 in the distal promoter. The numbering of nucleotides is relative to the transcription start site (+1).* ***B)*** *The nucleotide sequence of the rat BSP gene proximal promoter is shown from −201 to −35. Inverted CCAAT box, CRE, FRE, NFκB, Pit-1, AP-1, and HOX are present.*

mutation of the FRE (M-FRE) significantly reduced the amelogenin effect on the transcriptional activity (Fig. 6). These results suggest that the FRE is required as functional *cis*-elements for upregulation of BSP transcription by amelogenin.

Gel Mobility Shift Assays

To identify nuclear proteins that bind to binding elements in pLUC3, pLUC4, and pLUC5, double-stranded oligonucleotides were end-labeled and incubated with nuclear proteins (3 µg) extracted from confluent ROS 17/2.8 cells that were either not treated (control) or treated with 1 µg/ml amelogenin for 3 and 12 hours. With nuclear extracts from confluent control cultures of ROS 17/2.8 cells, shifts of HOX DNA-protein complexes (Fig. 7; lane 1) and a shift of a single FRE DNA-protein complex were evident (Fig. 7, lane 7). While HOX DNA-protein complexes did not change following stimulation by amelogenin (1 µg/ml) for 3 and 12 hours (Fig. 7; lanes 2 and 3), DNA binding activity to FRE was increased (Fig. 7; lanes 8 and 9). That the

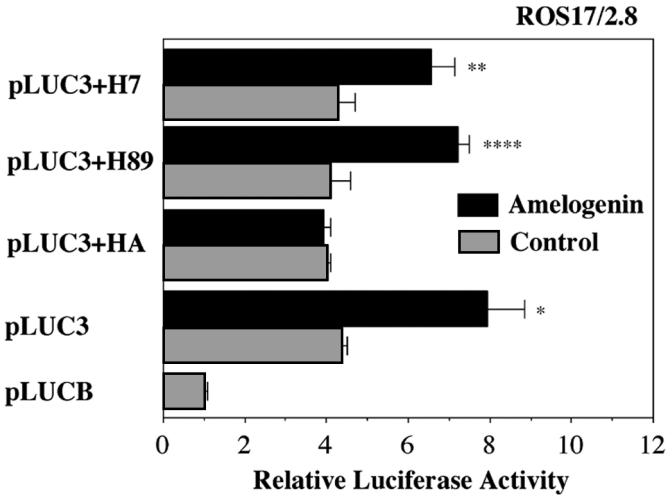

Figure 5.
Effect of kinase inhibitors on transcriptional activation by amelogenin. Transient transfection analysis of pLUC3 treated with amelogenin (1 µg/ml) for 12 hours in ROS17/2.8 cells is shown together with the effects of the PKC inhibitor (H7, 5 µM), PKA inhibitor (H89, 5 µM), and tyrosine kinase inhibitor (herbimycin A; HA, 1 µM). The results obtained from three separate transfections were combined and the values expressed with standard errors. Significant differences compared to controls are shown at the following probability levels: *P <0.1; †P <0.05; ‡P <0.01.

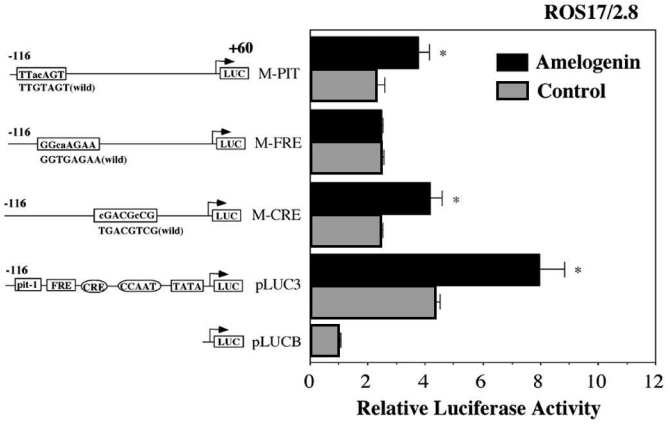

Figure 6.
*Site mutation analysis of luciferase activities. Dinucleotide substitutions were made within context of the homologous -116 to +60 (pLUC3) BSP promoter fragments. M-CRE (**c**GACG**c**CG), M-FRE (GG**ca**AGAA), and M-PIT (TT**ac**AGT) were analyzed for relative promoter activity after transfection into ROS 17/2.8 cells and examined for induction after treatment with amelogenin (1 mg/ml) for 12 hours. The results of transcriptional activity obtained from three separate transfections with constructs were combined and the values expressed with standard errors. Significant differences compared to controls are shown at the following probability levels:* *P <0.1.

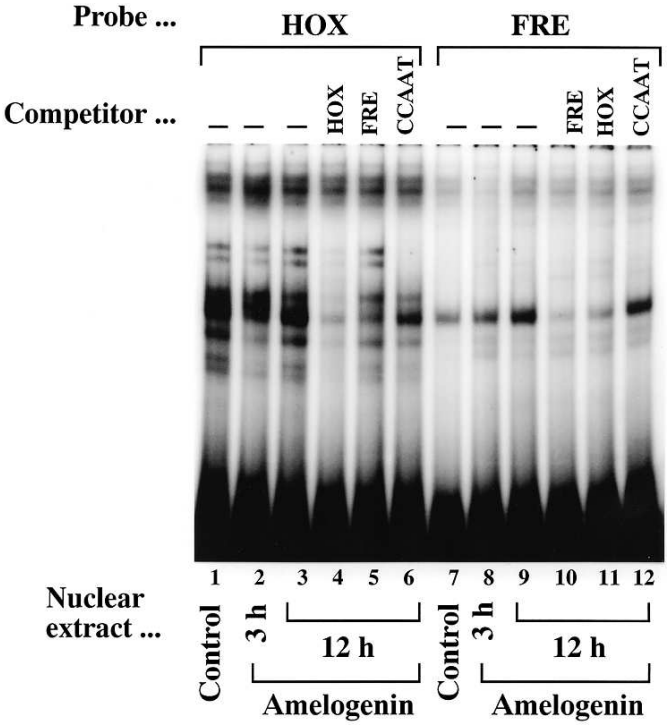

Figure 7.
*Amelogenin increases the DNA binding activity recognized by the FRE sequence. Radiolabeled double-stranded HOX (–204 TCCTCAGCCT **TCAATTAAA**TCCCACA –179; lanes 1 through 6) and FRE (98 TTTT CT**GGTGAGAA**CCCACA 79; lanes 7 through 12) oligonucleotides were incubated for 20 minutes at 21°C with nuclear protein extracts (3 μg) obtained from ROS 17/2.8 cells incubated without (lanes 1 and 7) or with amelogenin (1 μg/ml) for 3 hours (lanes 2 and 8) and 12 hours (lanes 3 through 6 and 9 through 12). Competition reactions were performed using a 40-fold molar excess of unlabeled HOX, FRE, and CCAAT. DNA protein complexes were separated on 5% polyacrylamide gel in low-ionic-strength tris-borate buffer, dried under vacuum and exposed to an imaging plate for quantitation using an imaging analyzer.*

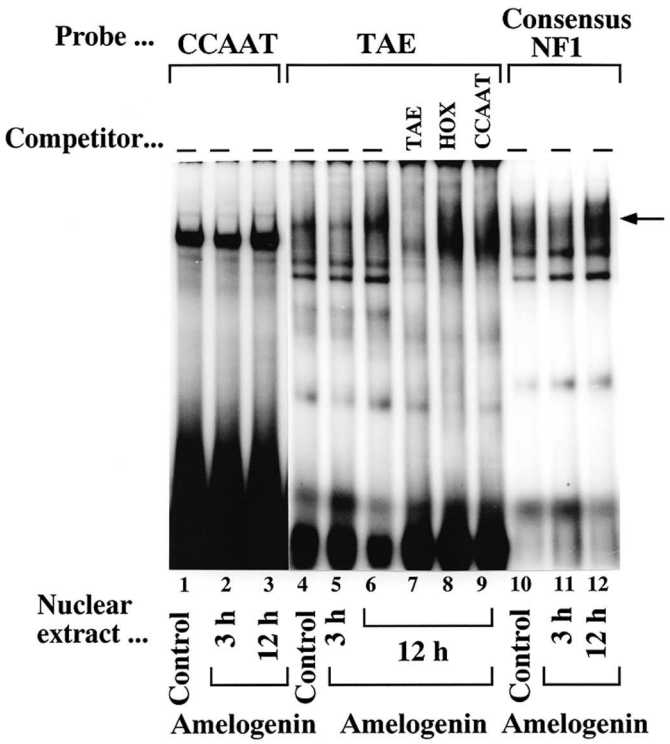

Figure 8.
*Amelogenin increases the DNA binding activity recognized by TAE sequence. Radiolabeled double-stranded inverted CCAAT (–61 CCGTGA CCGTG**ATTGG**CTGCTGAGA –37), TAE (–506 CAAAGCC**TTGGC**A GCCC**GGCTG**GCT –482), and consensus NF1 (CCT**TTGGC**AT GCT**GCCAA**TAT) oligonucleotides were incubated for 20 minutes at 21°C with nuclear protein extracts (3 μg) obtained from ROS 17/2.8 cells incubated without (lanes 1, 4, and 10) or with amelogenin (1 μg/ml) for 3 hours (lanes 2, 5, and 11) and 12 hours (lanes 3, 6 through 9, and 12). DNA protein complexes were separated on 5% polyacrylamide gel in low-ionic-strength tris-borate buffer, dried under vacuum, and exposed to an imaging plate for quantitation using an imaging analyzer.*

DNA protein complexes represent specific interactions were indicated by competition experiments in which a 40-fold excess of HOX and FRE reduced the amount of complex formation (Fig. 7; lanes 4 and 10). In contrast, CCAAT (40-fold) did not compete with complex formation (Fig. 7; lane 6 and 12). While 40-fold excess of the FRE slightly reduced HOX-protein complexes formation (Fig. 7; lane 5) and 40-fold excess of the HOX reduced FRE-protein complex formation (Fig. 7; lane 11). When we used the inverted CCAAT sequence as a probe, the DNA-NF-Y protein complex did not change after stimulation by amelogenin (Fig. 8; lanes 1, 2, and 3).[27,28] TAE was identified as containing the 5'-portion of the NF-1 canonical sequence (TTGGC) overlapping a putative AP-2 site.[35] When the TAE was incubated with the nuclear extract from control (no treatment) and amelogenin treatment (3 hours) cultures of ROS 17/2.8 cells, no significant binding was evident (Fig. 8; lanes 4 and 5). After stimulation by amelogenin (1 μg/ml) for 12 hours, DNA binding activity was increased (Fig. 8; lane 6). The TAE-protein complex representing specific interaction was indicated by competition experiments in which 40-fold excess of TAE reduced the amount of complex formation (Fig. 8; lane 7). In contrast, HOX and CCAAT (40-fold) did not compete with complex formation (Fig. 8; lanes 8 and 9). When we used consensus NF-1 as a probe, NF-1 DNA-protein complex increased after stimulation by amelogenin for 12 hours (Fig. 8; lane 12).

DISCUSSION

These studies have shown that amelogenin increases expression of BSP gene in osteoblastic ROS 17/2.8 cells and SBMC cells. Transduction of the amelogenin signaling is mediated through a tyrosine kinase, which targets nuclear proteins that bind to FRE and TAE elements in the proximal promoter of the BSP gene.

BSP is a unique marker of early osteogenic differentiation that can regulate the formation of mineral crystals.[18] Our results show that amelogenin treatment

of ROS 17/2.8 cells increases the steady-state level of BSP mRNA approximately 2.4-fold (Fig. 1). Amelogenin stimulated BSP promoter activity ~1.5-fold in pLUC3 and pLUC4, and ~2.3 fold in pLUC5 constructs (Fig. 2), which is comparable with the increases in BSP mRNA levels. These results suggest that amelogenin regulates BSP mRNA expression, at least in part, via transcriptional control. When we used SBMC cells for luciferase assays to see the effect of amelogenin on BSP transcription, amelogenin stimulated BSP transcription (Fig. 3). Therefore, amelogenin increases BSP expression not only in transformed ROS 17/2.8 cells but also in normal osteoprogenitors.

From transient transfection assays we initially located the amelogenin responsive region to the proximal promoter (pLUC3; nucleotides –116 to –43) of the BSP gene (Fig. 2), which encompasses an inverted CCAAT box (nucleotides –50 to –46), a cAMP response element (CRE; nucleotides –75 to –68), a FGF2 response element (FRE; nucleotides –92 to –85), and a Pit-1 (nucleotides –111 to –105) motif (Fig. 4). Luciferase activity of pLUC5 further enhanced by amelogenin from 1.5-fold (pLUC3 and pLUC4) to 2.3-fold, suggesting that an amelogenin response region exists not only in pLUC3 but also in pLUC5. Although mutations of the CRE (M-CRE) and Pit-1 (M-PIT) were without effect, the transcriptional stimulation by amelogenin was abrogated in the FRE mutation (M-FRE; Fig. 6). The involvement of the FRE element is further supported by gel shift analyses in which more complexes with the FRE element formed using nuclear extracts from amelogenin stimulated cell (Fig. 7). The nuclear factor binding to the FRE, which is regulated by tyrosine kinase, is the focus of current studies because of its potential role in regulating basal and FGF2-induced transcription of BSP in osteoblast,[31] as well as prostaglandin E_2 effects.[30] In this study the tyrosine kinase inhibitor, herbimycin A inhibited amelogenin-induced promoter activity (Fig. 5), indicating that amelogenin increases expression of the BSP gene through a tyrosine kinase pathway. The nuclear factors binding to the inverted CCAAT box and HOX did not change after stimulation by amelogenin. Very interestingly, HOX oligonucleotides can compete with FRE-protein complex formation (Fig. 7; lane 11), and FRE reduced HOX-protein complexes formation (Fig. 7; lane 5). The results might have been caused by 3'-portion of the FRE (T**GGTGAGAA***CCCACA*GC), which resembles the 3'-portion of the HOX sequence (T**TCAATTAA**AT*CCCACA*AT) (Fig. 4). The specific sequence in pLUC5 is between –425 and –801, and the identified response element in this region is the TAE. TAE was identified as TGF-β1 activation element which containing the 5'-portion of the NF-1 canonical sequence (TTGGC).[35] Results of gel shift assays show that TAE-protein complex and NF-1-protein complex were increased by amelogenin (1 μg/ml) for 12 hours (Fig. 8). These results indicate that amelogenin stimulates BSP expression through FRE and TAE elements in the BSP gene promoter. Further study is necessary to investigate transcription factors that bind to FRE and TAE, and the functional interactions of these elements.

In our previous study, EMD (50 μg/ml, 12 hours) increased luciferase activities of pLUC4 (nucleotides –425 to +60) and pLUC5 (nucleotides –801 to +60). EMD regulates BSP transcription through HOX and TAE elements in the BSP gene promoter.[11] Ninety percent of the protein in the EMD is amelogenin and the remaining 10% is non-amelogenin enamel matrix proteins and growth factors such as TGF-β and bone morphogenetic protein (BMP).[11] Discrepancy between the activities of EMD and amelogenin on BSP transcription might be caused by the complex components of EMD.

In this study, we have shown that amelogenin induced BSP gene expression in osteoblast-like ROS 17/2.8 cells and rat stromal bone marrow cells. Our results suggest that amelogenin can have a positive effect on periodontal regeneration, such as formation of new cementum and alveolar bone. Continued studies necessary to investigate amelogenin effects on periodontal ligament cells, which are crucial cell population for periodontal regeneration and will be aimed at defining the transcription factors that mediate the promotion of mineralization by amelogenin.

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Correspondence: Dr. Yorimasa Ogata, Department of Periodontology, Nihon University School of Dentistry at Matsudo, Chiba 271-8587, Japan. Fax: 81-47-360-9362; e-mail: ogata@mascat.nihon-u.ac.jp.

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