Sulfated Polysaccharides Enhance the Biological Activities of Bone Morphogenetic Proteins*

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Bone morphogenetic proteins (BMPs), which have been shown to be heparin-binding proteins, induce osteoblast differentiation in mesenchymal cells. In the present study, we examined the effects of heparin on the BMP activities in C2C12 myoblasts. Heparin dose dependently enhanced the osteoblast differentiation induced by not only homodimers of BMP-2 or BMP-4 but also heterodimers of BMP-2/6 or BMP-2/7. However, the osteoblast differentiation induced by the constitutively active BMPR-IA, a functional BMP type I receptor, was not affected by heparin. Heparan sulfate and dextran sulfate also enhanced the BMP-2 activity, although the chemically desulfated heparin-derivatives have lost this stimulatory capacity. Heparin dose-dependently suppressed the accumulation of BMP-2 from the culture media into the cell layer or BMPR-IA, and retained a large amount of BMP-2 in the culture media. The biological activity of BMP-2, which was evaluated using a BMP-responsive reporter gene expression, was prolonged in the presence of heparin. Taken together, these results suggest that sulfated polysaccharides enhance the biological activity of both homodimers and heterodimers of BMPs by continuously serving the ligands to their signaling receptors expressed on cell membranes.

Bone morphogenetic proteins (BMPs)† were originally identified as unique in demineralized bone matrix that could induce ectopic bone formation when implanted into muscular tissues (1, 2). More than 15 members of BMPs have been identified, and they are members of the TGF-β superfamily (3–7). They are classified into several BMP subfamilies based on their homology within the mature domains; the BMP-2 and BMP-4 subfamily, the BMP-5, BMP-6, BMP-7 (also called OP-1) and BMP-8 subfamily, the GDF-5, GDF-6 (BMP-13), and GDF-7 (BMP-12) subfamily, and the BMP-3 and GDF-10 (BMP-3b) subfamily (3–7). Most BMPs are first synthesized in large inactive preproproteins. They form homodimers or heterodimers via a disulfide bond in the mature domain, and then are secreted as active dimers after proteolytic processing (3–7). Several recombinant proteins of BMP dimers were reported to be active in an ectopic bone formation assay. Some heterodimers were shown to be more potent than each homodimer (8–11). Several lines of evidence suggest that BMPs are key molecules for normal skeletal development in vertebrates (3, 5–7, 12). We previously reported that BMP-2 inhibits myogenic differentiation of C2C12 myoblasts, and converts their differentiation pathway into that of osteoblast lineage cells (13). Both the ectopic bone-inducing activity and the osteoblast differentiation-inducing activity appear to be unique for BMPs among members of the TGF-β superfamily.

Signaling of BMPs is initiated by binding to the specific transmembrane receptors, type I and type II serine/threonine kinase receptors (6, 12, 14–16). The type I receptors are activated by the ligand bound-type II receptors, and then phosphorylated Smad proteins as substrates in the cytoplasm. The phosphorylated Smad forms a complex with Smad4, translocates into the nucleus, and regulates the gene expression in cooperation with other transcription factors. Recently, a GC-rich sequence was identified as a Smad1/Smad4-binding element in the 5′-upstream region of the Id1 gene, which is one of the target genes of BMPs (17–19). Constitutively active BMP type I receptors can activate BMP signaling in the absence of ligands (20, 21). In contrast, dominant-negative BMP type I receptor, in which the kinase domain was truncated, blocked the response to BMPs (22). Using these mutant receptors, BMPR-IA was identified as a functional type I receptor for BMP-2 in C2C12 cells (22).

A large number of extracellular molecules were reported to regulate positively and negatively the biological activities of BMPs in vivo and in vitro (6, 7, 12). Sulfated polysaccharides such as heparin and heparan sulfate are macromolecules associated with the cell surface and the extracellular matrix (23–28). These polysaccharides have been shown to interact directly with a number of growth factors, including BMPs, via highly negative charged polysaccharide chains. Indeed, heparin-affinity chromatography was used to purify the BMP activity from extracts prepared from demineralized bone matrix (29, 30). Heparin enhances the anticoagulation and the growth promoting activities of antithrombin III and FGFs, respectively (31–34), and the anticoagulation activity of heparin is widely used clinically. We report here that sulfated polysaccharides includ-
ing heparin, heparan sulfate and dextran sulfate, but not desulfated heparin, enhance the osteoblast differentiation induced by homodimers and heterodimers of BMPs. Heparin reduced the amounts of BMP-2 bound to the cell layer or its receptor and maintained that level in the culture medium. The biological activity of BMP-2 was prolonged in the presence of heparin. Taken together, these results suggest that sulfated polysaccharides enhance the biological activity of BMPs by continuously serving the ligands to their signaling receptors expressed on the cell membranes.

MATERIALS AND METHODS

BMPs and Polysaccharides—Purified recombinant human BMP-2 and BMP-4 were obtained from Yamanouchi Pharmaceuticals Co., Ltd. (Tokyo, Japan) and Genzyme (Cambridge, MA), respectively. Conditioned media of COS7 cells containing BMPs were prepared as described below. Heparin prepared from porcine intestine, heparan sulfate prepared from bovine intestine and kidney, synthetic dextran sulfate with different molecular weights (5,000 (DS 0.5), 10,000 (DS 1.0), and 500,000 (DS 50)) were purchased from Sigma Chemical Co. Chemically modified heparin derivatives, CDSNAc, CDSNS, and NDSNAc, were purchased from Seikagaku Co. (Tokyo, Japan). Plasmids—Human BMP-2, BMP-4, BMP-6, and BMP-7 cDNAs and mouse ALP-FLAG cDNA were cloned from human SaOS-2 osteosarcoma cells and mouse C2C12 cells stimulated with BMP-2 by RT-PCR using appropriate primers; 5'-ATGTAATCATGTCGAGCCCGGGCCGCTGG-3' (BMP2-S) and 5'-TTCTAGACTAGCGACACCCACAACCCT-3' (BMP2-AS), 5'-CAAGAACACCATGTCTGTTG-3' (BMP4-S) and 5'-TTCTAGATCAAGCCGGCCGGGACGCC-3' (BMP4-AS), 5'-CCGGTATCGGCGGGGCGGGCGCGCC-3' (BMP6-S) and 5'-TTCTAGATCAAGCCGGGCAGTGGCC-3' (BMP6-AS), 5'-GAGGCGGGGCAGGTCAGCTG-3' (BMP7-S), and 5'-GGCTTACATCTCTCAGAGGAGG-3' (BMP7-AS), and 5'-TTGAATTTCCGCGGAGGACATCTCAGG-3' (ALP-S) and 5'-TTCTAGATCAGCTGACTTACGTCTGCTGCTTGTGTCAGCAGGACGTCG-3' (ALP-FLAG-S). The BMP cDNAs and the ALP cDNA obtained from the cell membranes. The FLAG-BMPR-IA-His complex was immunoprecipitated with an anti-FLAG antibody conjugated agarose (Sigma) for 15 h at 4°C. The immunoprecipitates were separated in 12% SDS-PAGE gel, and analyzed with Western blotting using an anti-His antibody (H15) (Santa Cruz Biotechnology, Inc.). The primary antibody was detected using a horseradish peroxidase-conjugated secondary antibody and the ECL plus Western blotting detection reagents (Amersham Biosciences).

Monitoring of BMP-responding Cells—C2C12 cells were transiently transfected with pI985-EGFP/p224 plasmid (19) and treated with BMP-2 in the presence or absence of 4 μg/ml of heparin as described above. EGFP (+) cells were monitored using a BX50 fluorescent microscope with a DP50 digital CCD camera (Olympus, Tokyo, Japan).

RESULTS

Heparin Enhances the BMP-induced Osteoblast Differentiation in Myoblasts and Osteoblast Lineage Cells—First we examined the effect of heparin on the BMP-induced cell differentiation in C2C12 myoblasts, which was evaluated histochemically based on ALP activity and MHC expression as markers for osteoblast and myogenic differentiation, respectively (Fig. 1A). BMP-2 inhibits the MHC expression and induces the ALP activity at 100 ng/ml or greater in C2C12 cells. BMP-4 also showed similar effects in these cells (Fig. 1A). Treatment cells with 100 or 300 ng/ml of BMP-2 or BMP-4 in the presence of heparin markedly increased the number of ALP (+) cells. This stimulatory capacity of heparin on the ALP activity induced by BMP-2 was dose-dependent up to 30 μg/ml (Fig. 1B). Moreover, heparin also enhanced the BMP-2-induced suppression of the MHC expression in a dose-dependent manner (Fig. 1C). In both cases, ED50 of heparin was roughly estimated at 4 μg/ml. Heparin itself did not show significant induction of the ALP activity or the inhibition of the MHC expression up to 30 μg/ml (Fig. 1, A–C). The expression of osteocalcin mRNA, another marker for osteoblast differentiation, was induced by BMP-2 at 300 ng/ml in the absence of heparin. These levels of osteocalcin mRNA were increased by heparin in a dose-dependent manner (Fig. 1D).

Measurement of Sulfur in Polysaccharides—The amount of sulfur in polysaccharides was measured as described previously (35). In brief, the polysaccharide was hydrolyzed with 2 M hydrochloric acid for 5 h at 100°C. Then trichloroacetic acid was added and further incubated with barium chloride-gelatin reagent at room temperature for 20 min. The extinction of the solution was measured at 500 nm.

Transfection and Preparation of Conditioned Media Containing BMPs—C2C12 cells were inoculated into 96-well plates at a density of 1.5 × 10^4 cells/well 1 day before transfection, and they were transiently transfected with 0.2 μg/well of DNA and 0.75 μg/well of a cationic liposome reagent (LipofectAMINE 2000, Invitrogen) according to the manufacturer’s protocol. Cells were incubated for 3 h with the DNA-liposome complex, then the culture media were changed to DMEM containing 2.5% FBS with or without 4 μg/ml of heparin, and cultured for an additional 3 days. Cells were fixed and histochemically stained for ALP as described previously (13). To prepare condition media containing BMPs, COS7 cells were inoculated in 6-well plates at 4.5 × 10^5 cells/well in DMEM containing 15% FBS 1 day before transfection. Cells were transfected with 1 μg/well of DNA, 4 μg/well of LipofectAMINE and 6 μg/well of Plus reagent according to the manufacturer’s protocol. After overnight incubation, the culture media were changed to DMEM containing 2.5% FBS and further cultured for an additional 2 days. Conditioned media were recovered from the cultures, and cell debris and other precipitates were removed by centrifugation. The conditioned media were diluted to 50% with fresh DMEM containing 2.5% FBS and added to the C2C12 cell cultures.

Immunoprecipitation and Western Blots—For the immunoprecipitation and Western blotting experiments, FLAG-BMP-2 was produced for 2 days in the conditioned media of COS7 cells. C2C12 cells were transiently transfected with ΔBMPRIA-IA by LipofectAMINE 2000 as described above, and cultured for an additional 2 days in DMEM at 15% FBS. The cells were incubated for 2 h at 4°C with the conditioned media containing FLAG-BMP-2 in the presence or absence of 4 μg/ml of heparin. The cell layer was rinsed with phosphate-buffered saline containing 0.9 mM CaCl2 and 0.49 mM MgCl2 (PBS (+)), and then the cell surface components were cross-linked with 1 μM BS3 (Pierce) in PBS (+) for 15 min at 4°C. The cell layer was lysed in TBS buffer (25 mM Tris-HCl, pH 7.5, and 150 mM NaCl) containing 1% Triton X-100 and a 1× protease inhibitor mixture (Roche Applied Science). The FLAG-BMP-2 and ΔBMPRIA-IA-His complex was immunoprecipitated with an anti-FLAG antibody conjugated agarose (Sigma) for 15 h at 4°C. The immunoprecipitates were separated in a 12% SDS-PAGE gel, and analyzed with Western blotting using an anti-His antibody (H15) (Santa Cruz Biotechnology, Inc.). The primary antibody was detected using a horseradish peroxidase-conjugated secondary antibody and the ECL plus Western blotting detection reagents (Amersham Biosciences).
ing that C2C12 cells do not express BMPs even in the presence of heparin. Heparin gradually suppressed the proliferation capacity of C2C12 cells up to 30 μg/ml, but no significant difference was observed in the presence and absence of BMP-2 (Fig. 1E). The stimulatory effect of heparin on the osteoblast differentiation induced by BMP-2 was also observed in the bone marrow-derived stromal cell line, ST2, and osteoblastic cell line, MC3T3-E1 (Fig. 1F).

Heparin Stimulates Biological Activity of Homodimers and Heterodimers of BMPs—BMPs act as dimers consisting of monomers that belong to the same or different BMP subfamily. We examined the activity of homodimers and heterodimers of BMP-2, BMP-4, BMP-6, and BMP-7 by cotransfecting each in C2C12 cells, and their activity was evaluated by ALP staining after 3 days. As shown in Fig. 2A, transfection of BMP-2, BMP-4, BMP-6, or BMP-7 alone induced a few ALP (+) cells. In contrast, cotransfection of BMP-2 and BMP-6, BMP-2 and BMP-7, BMP-4 and BMP-6, and BMP-4 and BMP-7 markedly induced the ALP (+) cells (Fig. 2A). To examine whether BMPs act as homodimers or heterodimers in these cultures, we prepared conditioned media from COS7 cells transfected with BMP-2, BMP-6, BMP-7, BMP-2/BMP-6, and BMP-2/BMP-7. Again, the conditioned media cotransfected BMP-2/BMP-6 or BMP-2/BMP-7 strongly induced the ALP activity in C2C12 cells (Fig. 2, B and C). In contrast, mixed conditioned media of BMP-2, BMP-6 or BMP-7, which were prepared individually and combined immediately before treatment of the cells, showed no significant synergism, suggesting that only heterodimers of BMPs have higher activity. Heparin also increased the number of ALP (+) cells induced by BMP-2/BMP-6 or BMP-2/BMP-7 (Fig. 2, B and C). However, heparin failed to increase the number of ALP (+) cells in the cultures treated with conditioned media of BMP-6 or BMP-7 (Fig. 2, B and C).

Other Types of Sulfated Polysaccharide Also Enhance the BMP Activities—To determine the structural features of the heparin, we examined the structurally related but less sulfated
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Fig. 2. Heparin enhances the biological activities of homodimers and heterodimers of BMPs. A, cotransfection of BMPs in C2C12 cells synergistically increased the osteoblast differentiation-inducing activity. C2C12 cells were cotransfected BMPs in various combinations as indicated in the figure, and cultured for 3 days. Cells were histochemically stained for the ALP activity. (B and C) Heparin enhances the biological activity of heterodimers BMP-2/6 and BMP-2/7. BMP-2, BMP-6, and BMP-7 were transfected individually or together in COS7 cells, and the conditioned media were prepared. C2C12 cells were treated with these conditioned media in the presence or absence of 4 μg/ml of heparin for 3 days. The ALP activity was histochemically stained in B and quantified in C.

polysaccharide, heparan sulfate, prepared from intestine or kidney. As shown in Fig. 3, A and B, only intestine-derived heparan sulfate showed a stimulatory capacity on the BMP-2 activity in C2C12 cells. However, the ALP activity enhanced by the intestine-derived heparan sulfate was significantly lower than heparin (Fig. 3B). Next, we examined the effects of chemically modified heparins on the BMP-2-induced cell differentiation in C2C12 myoblasts. In this experiment, three heparin derivatives, CDSNAc, CDSNS, and NDSNAc, were compared with heparin at 4 μg/ml. All of these derivatives almost completely lost the stimulatory capacity on the BMP-2-induced ALP activity (Fig. 3, C and D), suggesting that both the O- and N-sulfate groups of heparin play important roles in the stimulatory activity. We further examined the effects of 3 types of dextran sulfate, which are synthetic sulfated polysaccharides, on the biological activity of BMP-2. High MW dextran sulfate (DS 50) increased the ALP activity at 0.1 μg/ml and low MW dextran sulfate molecules (DS 1.0 or DS 0.5) increased it at 1.0 μg/ml or greater (Fig. 3, E and F). However, treatment with dextran sulfate alone failed to induce the ALP activity (Fig. 3F). Next, we measured the total amounts of sulfur in the polysaccharides. Although heparin and 3 types of dextran sulfate contained 250 μg of sulfur mg⁻¹ of polysaccharide, CDSNAc contained less than 15 μg of sulfur mg⁻¹ of polysaccharide (Fig 3G). Other types of polysaccharides examined, including kidney- and intestine-derived heparan sulfate, CDSNS, and NDSNAc, contained sulfur greater than 50% heparin.

Molecular Mechanism of the Stimulatory Effect of Heparin on BMP Activity—Next, we examined the molecular mechanism of heparin in the BMP signaling pathway. For this purpose, we examined the effects of heparin on the ALP activity induced by the different conditions in C2C12 cells. As shown in Fig. 4, heparin failed to stimulate the ALP activity in the cultures transfected with the expression vector of BMP-2 or BMPR-Iα alone or the existively active BMP receptor and Smad1. In contrast, heparin clearly increased the number of ALP (+) cells induced by transfection of a BMP-2 expression vector in C2C12 cells, suggesting that heparin affects primarily BMPs rather than intracellular signaling or the ALP enzyme (Fig. 4).

To examine the effect of heparin on the BMP-receptor binding, we generated FLAG-BMP-2, in which a FLAG epitope sequence was inserted in the mature domain, and then transfected into COS7 cells. FLAG-BMP-2 was recovered in the conditioned media and also stimulated the ALP activity in C2C12 cells (Fig. 5 and data not shown). We examined the amounts of complex consisting of FLAG-BMP-2 and ABMPR-Iα receptor in C2C12 cells in the presence and absence of heparin. Surprisingly, heparin decreased the amounts of the ligand-receptor complex in a dose-dependent manner (Fig. 5A). Similar results were obtained using constitutively active BMPR-Iα-His (data not shown). It was noticed that the amounts of FLAG-BMP-2 bound to the cell/extracellular matrix fraction, which were detected by Western blotting using anti-FLAG antibody without immunoprecipitation, were also decreased by heparin (Fig. 5A). Thus, we monitored the amounts of FLAG-BMP-2 in the culture media. As shown in Fig. 5B, FLAG-BMP-2 disappeared from the culture media within 48 h in the absence of heparin. In the presence of heparin, however, a significant amount of FLAG-BMP-2 was still detected at 48 h (Fig. 5B).

Finally, we examined the effects of heparin on the BMP signaling using an EGF reporter, Id985-EGFPd2, in which a destabilized EGF was driven under the control of a BMP-responsive element in the human Id1 gene (19). When the Id985-EGFPd2 plasmid was transfected into C2C12 cells and treated with BMP-2 in the absence of heparin, the EGFP (+) cells transiently appeared at 4 h but disappeared at 30 h (Fig. 5C). Heparin did not increase the number of the EGFP (+) cells...
at 4 h. However, large numbers of EGFP (+) cells were observed in the cultures treated with BMP-2 in the presence of heparin (Fig. 5C).

DISCUSSION

In the present study, the effects of heparin on the biological activities of homodimers and heterodimers of BMPs were examined. We found that not only heparin but also heparan sulfate and dextran sulfate enhanced the biological activities of BMPs. The glypican family proteoglycans, which contain heparan sulfate polysaccharide chains, have been reported to play important roles in the BMP signaling in vertebrates and flies (37). Recently, loss of the function mutation in glypican-3 was identified as the mutation responsible for Simpson Golabi Behmel syndrome in humans and mice, in which polydactyly and other skeletal abnormalities were observed (38, 39). Taken together, these findings suggest that highly sulfated polysaccharides such as heparin and heparan sulfate physiologically regulate the BMP activities in vivo. It was suggested that sulfated polysaccharide chains are essential for the stimulatory capacity because desulfated heparin-derivatives have lost it. However, the total amount of sulfur in the chains did not parallel the capacity among various types of polysaccharides. These results suggest that not only the total amounts of sulfur but also its position and/or structure is important to modify the stimulatory capacity of BMP activity. The basic amino acid stretch found in the BMP-2 and BMP-4 subfamily may be involved in the interaction with the negatively charged polysaccharides (discussed below). It was also suggested that clinical application of the sulfated polysaccharides with BMPs locally stimulates their bone-inducing activity in vivo. This possibility is currently under investigation in our laboratories.

Both heparin and heparan sulfate enhance the biological activities of FGFs. In these cases, the polysaccharides were reported to act as co-receptors and stimulate ligand binding to the signaling receptors (33, 34). As shown in the present study, heparin also did not primarily affect the intracellular signaling of BMPs. Thus, it was initially suggested that heparin also acts as a co-receptor in this case. However, heparin decreased the

Fig. 3. Other types of sulfated polysaccharides also enhance the BMP activities in C2C12 cells. C2C12 cells were cultured for 3 days without or with 100 ng/ml BMP-2 in the absence and presence of heparan sulfate (A and B), chemically modified heparin derivatives (C and D), or synthetic dextran sulfate (E and F). Then the ALP activity was stained histochemically (A, C, and E) or measured using p-nitrophenylphosphate as a substrate (B, D, and F). The concentrations of polysaccharides used were 4 μg/ml of heparin and heparan sulfate in A, 4 μg/ml of heparin-derivatives in C and D, and the others are indicated in each figure. G, total amounts of sulfur in various types of polysaccharides were measured as described under “Materials and Methods.” Values are means ± S.E. of three samples.
BMP-2 binding to the functional receptor in the immunoprecipitation and Western blotting experiments, suggesting that another novel mechanism is involved in this case. We found that heparin retained the amounts of BMP-2 in the culture media and suppressed the accumulation of BMP-2 into the cell/extracellular fraction. To examine the biological activity of BMP-2 retained in the media, we used an EGFP reporter gene, Id985-EGFPd2, in which a destabilized EGFP was driven under the control of a BMP-2 responsive element in the human Id1 gene (19). In agreement with the results in the ligand-receptor binding assay, we did not see any increase in the number of EGFP (+) cells even in the presence of heparin at 4 h after treatment. However, large numbers of the EGFP (+) cells were observed only in the presence of heparin at 30 h. These results clearly suggest that BMP-2 retained in the culture media by heparin had not lost its biological activity and was still capable of binding to the signaling receptors. Crystal structural analysis of BMPs and their receptors suggested that the conserved region in the mature domain of BMPs over seven cysteine residues is critical for binding to the receptors (40–42). In contrast, the basic amino acid stretch at the amino-terminal region, which is located outside this receptor-binding domain, and conserved only in the BMP-2 and BMP-4 subfamily, was suggested as the heparin-binding site in this BMP subfamily (23, 43). Thus, heparin bound to BMPs may not prevent their binding capacity to the receptors. We previously reported that continuous stimulation by BMP-2 was required for the osteoblast differentiation in C2C12 cells (13). Moreover, in our preliminary experiments, frequently changing the culture media to maintain the BMP concentration in the media markedly stimulated the BMP-induced osteoblast differentiation of C2C12 cells as in the case of the treatment with heparin. Taken together with the present results, it is suggested that heparin enhances the BMP activities by making complexes with BMPs in the culture media to prevent their accumulation into the cell/extracellular fraction, and continuously serving the ligands to their signaling receptors.

Recently, it was reported that exogenous BMP-2 and TGF-β were internalized by endocytosis after binding to their specific receptors expressed on the cell surfaces (44–46). The BMP-2 activity, including the induction of ALP activity, was enhanced in C2C12 cells when the cells were treated with inhibitors for endocytosis or a hyposmotic condition (46). These results suggest that not only the binding affinity of a ligand to the receptor but also stability of the ligand-receptor complex on the cell surface is one of the key factors to control the biological activity of the ligand in the target cells. It is also suggested that the sulfated polysaccharides inhibit endocytosis of BMP-2 in C2C12 cells. Further studies are necessary to clarify further the molecular mechanisms of the stimulatory capacity of the sulfated polysaccharides on the BMP activity.

BMPs were originally purified from crude extracts of demineralized bone matrix using heparin-affinity chromatography (29, 30). Although the amino acid sequences of several BMPs have been identified in active fractions, it is still unclear that BMPs act physiologically as homodimers or heterodimers. In
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the developing tissues during embryogenesis, some BMP transcripts were expressed in the overlapping regions (47–49). Moreover, crossing heterozygous BMP mutant mice to generate a doubly heterozygous strain showed more severe skeletal abnormalities than that of each single mutation (50, 51). Some of the recombinant BMP heterodimers such as BMP-2/6, BMP-2/7, and BMP-4/7 were shown to have more potent bone-inducing activity in vitro than their homodimers (8, 9). To our knowledge, this is the first study showing that BMP-4/6 also has potent bone-inducing activity. This hypothesis is further supported by the finding that cotransfection of two types of the same BMP subfamilies did not cause any synergism, at least in the present assay system. Further studies are required to elucidate the molecular mechanisms of the differences in the biological activities between homodimers and heterodimers of BMPs.

In conclusion, we found that heparin and heparan sulfates enhance the biological activities of homodimers and heterodimers of BMPs. These sulfated polysaccharides form complexes with BMPs via negatively charged polysaccharide chains and a basic amino acid stretch in BMPs in the culture media, and continuously serve the ligands to their signaling receptors.

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