Modulation of mitogenic activity of fibroblast growth factors by inorganic polyphosphate*

Toshikazu Shiba‡#, Daisuke Nishimura¶, Yumi Kawazoe¶, Yuichiro Onodera¶, Kaori Tsutsumi¶, Rie Nakamura¶, Minako Ohshiro¶

From the ‡ Frontier Research Division, Fujirebio Inc., 51, Komiya, Hachioji, Tokyo 192-0031, Japan.
¶ Division of Molecular Chemistry, Graduate School of Engineering, Hokkaido University, Sapporo 060-8628, Japan.

*This work was supported by Grant-in-Aid for Innovations through Business-Academic-Public Sector Cooperation and Grant-in-Aid for Scientific Research on Priority Areas (B) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

#To whom correspondence should be addressed.

E-mail: tz-shiba@fujirebio.co.jp
TEL & FAX: +81-426-45-4755

Running title: Modulation of FGF by poly(P)
The proliferation of normal human fibroblast cells was enhanced by the addition of inorganic polyphosphate [poly(P)] into culture media. The mitogenic activities of acidic fibroblast growth factor (FGF-1) and basic fibroblast growth factor (FGF-2) were also enhanced by poly(P). A physical interaction between poly(P) and FGF-2 was observed, and FGF-2 was both physically and functionally stabilized by poly(P). Furthermore, poly(P) facilitated the FGF-2 binding to its cell surface receptors. Since poly(P) is widely distributed in mammalian tissues, it may be a spontaneous modulator of FGFs.

Inorganic polyphosphates [poly(P)] are linear polymers of many tens or hundreds of orthophosphate residues linked by high-energy phosphoanhydride bonds and which have been found in a wide range of organisms, including bacteria, fungi, algae, mosses, insects, and protozoa, and in the tissues of higher plants and animals (1-4). The biological functions of poly(P) have been investigated mostly in microorganisms, and the following functions have been proposed: (i) storage substance of energy or orthophosphate, (ii) chelator of metal cations, (iii) donor for sugar and adenylate kinase, (iv) buffer against alkaline stress, (v) structural element in competence for DNA entry and transformation, and (vi) a regulatory factor of gene expression (1-4). Although the presence of poly(P) has been demonstrated in the rat brain, rat liver, human peripheral blood mononuclear cells, human erythrocytes, human gingival
fibroblasts, human osteoblasts and human plasma, and intracellularly in the nucleus, the mitochondria, lysosomes and plasma membrane (5), little is known about the functions of poly(P) in and the effects of poly(P) on mammalian cells. Recently, involvement of poly(P) in apoptosis and in modulation of the mineralization process in bone tissue (5, 6) have been suggested.

Since there has been no report concerning the direct effect of poly(P) on mammalian cells, and since poly(P) is widely distributed in mammalian tissues and plasma (5), we speculated that poly(P) has some physiological effect on cells. Based on this idea, we first studied in this report the effect of poly(P) on mammalian cell growth or proliferation \textit{in vitro} and revealed the novel poly(P) functions concerning modulation of mitogenic activity of fibroblast growth factors (7, 8).

**EXPERIMENTAL PROCEDURES**

\textit{Materials}-Normal dermal fibroblasts (NHDF), isolated from adult human, were purchased from BioWhittaker, Inc. (Maryland, USA). Normal human gingival fibroblasts (HGF), isolated from adult human, were provided by Dr. Nishimura (Osaka Dental University). Balb/c 3T3 cells were from Riken Cell Bank (Tsukuba, Japan). Poly(P) type 65 (sodium salts; with average chain length of 65 phosphate residues) was purchased from Sigma. Concentration of poly(P) are given in terms of phosphate residues. As the control of poly(P),
Na-PO$_4$ buffer (orthophosphate) was used. The pH of the Na-PO$_4$ buffer was adjusted to 7.0 by mixing the same concentrations of Na$_2$HPO$_4$ and NaH$_2$PO$_4$ solution. MTS cell proliferation assay kit was from Promega. Human recombinant FGF-2 was from Toyobo (Japan). Anti FGF-2 antibody was from Santa Cruz Biotechnology, Inc. (USA).

Assay for Cell Proliferation - Cells were seeded to 96 multiwell plates at 5x10$^3$ cells/well (100 µl/well) and cultured in Eagle’s minimal essential medium (E-MEM) (for NHDF) or Dulbecco’s modified Eagle’s minimal essential medium (D-MEM) (for Balb/c 3T3) containing 10 % FBS for 24 hours. After cells had adhered, the medium was replaced with E-MEM or D-MEM without FBS, and cells were further incubated for 48 hours. The medium was replaced again with an appropriate media described in the text and figure legends. After incubation at 37 °C, cell number was directly counted using hematocytometer after trypsinization, or was evaluated by MTS assay (9). For MTS assay, the medium was replaced with 100 µl of E-MEM (without Phenol Red), and 25 µl of mixture of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) (Promega) and phenazine methosulfate (PMS) solution (2 µg/ml MTS, 0.92 µg/ml PMS) was added to each well. After incubation for 75 min at 37 °C, the absorbance at 490 nm of each well was measured. The cell number was quantified by means of the bio-reduction activity of viable cells.

Preparation of Short-chain [¥P]-poly(P) - Long chain [¥P]-poly(P) was synthesized by using
purified *E. coli* polyphosphate kinase and purified as described (10). To prepare short-chain [32P]-poly(P) (average chain length of around 65 phosphate residues), the long-chain [32P]-poly(P) was hydrolyzed by 20 mM HCl for 2 min. at 95 °C, and the hydrolyzed poly(P) was separated by 15% polyacrylamide gel electrophoresis together with poly(P) type 65 as a marker. The conditions of gel electrophoresis were the same as those described in the legend of Fig. 3. The gel was stained by 0.05% toluidine blue containing 5% glycerol. The portion of the gel corresponding to the position of poly(P) type 65 was cut, and short-chain [32P]-poly(P) was isolated from the gel. Isolated gel was soaked in H2O and shaken at room temperature for 2 hours. After brief centrifugation, the supernatant was collected, and short-chain [32P]-poly(P) was recovered from the gel.

**Assay for binding between FGF-2 and high-affinity surface receptors**- The assay procedure was basically followed as described (11). Confluent Balb/c 3T3 cells were plated onto 60-mm dishes at 35,000 cells/cm² in D-MEM with 10% FBS at 37 °C. After 24 hours, the medium was changed to D-MEM with 2% FBS with 50 mM sodium chlorate to remove heparin sulfate proteoglycan (HSPG). Cells were further incubated for 72 hours at 37 °C and were then washed once with a binding buffer (D-MEM, 25 mM HEPES) at 4 °C. A fresh binding buffer was added (4 ml/well), and cells were incubated at 4 °C for 10 min. FGF-2 was added at the indicated concentrations, and cells were incubated at 4 °C for 2.5 hours. At the end of the binding period, cells were placed on ice and washed three times with ice-cold binding buffer. Cell
surface receptor-bound FGF-2 was extracted with two washes (one 5-min wash and one rapid wash, 0.5 ml each/dish) at room temperature using 2 M NaCl in 20 mM sodium acetate (pH 4.0). Washed solutions (0.5 ml x 2) containing the extracted cell surface receptor-bound FGF-2 were collected, and 5 ml of 8% bovine serum albumin was added to each sample as a carrier protein. Trichloroacetic acid was added to the final concentration of 5%, and precipitated proteins were pelleted by centrifugation. After removal of the supernatant, the protein pellet was neutralized by 1 N KOH and dissolved by SDS-PAGE sample buffer. FGF-2 was separated by 15% SDS-PAGE and visualized by western blotting.

RESULTS

Stimulation of Cell Growth by Adding Poly(P) into Culture media - Intriguingly, poly(P) stimulated the proliferation of both NHDF and HGF cells in the absence of serum (Fig. 1A and 1B). NHDF and HGF was grew continuously after 25 to 110 hours of incubation with poly(P), whereas there was almost no growth of cells incubated with Na-PO₄ buffer or with media only. In order to rule out the possibility that poly(P) enhances the reduction activity of mitochondria, resulting in the higher value of absorbance as measured by the MTS method (9), regardless of the cell number, cell growth was monitored by counting the number of cells. The number of viable cells after 3 days of incubation is
shown in Fig. 1C. In agreement with the result of measurement by the MTS method, the number of cells increased by approximately 2.8-folds after 72 hours of incubation with poly(P). However, no proliferation of cells incubated with media containing Na-PO₄ buffer was observed.

**Enhancement of Mitogenic Activity of FGFs by Poly(P)** – One possible mechanism of growth stimulation by poly(P) is that poly(P) enhances the activity of growth factors that were released into the culture medium from the cultured cells themselves. Therefore, in this study, we focused on the possibility of modulation of FGF activity by poly(P). Since FGFs have been implicated as autocrine growth factors (12), it is possible that poly(P) somehow modulates the biological activity of FGFs.

If poly(P) does in fact modulate the mitogenic activity of FGF, then cell proliferation would be enhanced by co-treatment with poly(P) and FGF rather than by single treatment with poly(P) or FGF. To examine whether the co-treatment of poly(P) and FGF is effective in stimulating mitogenic activity of FGFs, Balb/c 3T3, NHDF and HGF, whose growth could be dependent on FGF, were treated with FGF-1 or FGF-2 in combination with poly(P). As shown in Fig. 2, cell growth was slightly stimulated by both FGF-1 and FGF-2, and, moreover, the levels of growth stimulation by both FGFs became greater in the presence of poly(P). Cell growth in the medium containing FGF-1 and poly(P) was 1.6 to 1.9-times higher than that in the medium containing only FGF-1.
Similarly, cell growth in the medium containing FGF-2 and poly(P) was 1.3 to 1.7-times higher than that in the medium containing only FGF-2. Since the levels of growth stimulation by co-treatment of poly(P) and FGFs is greater than sum of stimulation levels of single treatment with poly(P) and FGFs in Balb/c 3T3 and NHDF, poly(P) seems to augment the mitogenic activities of FGFs. However, in HGF, the level of growth stimulation by co-treatment with FGF-2 and poly(P) is not greater than sum of stimulation levels of single treatment with poly(P) and FGFs. From these results, it is difficult to rule out the possibility that poly(P) and FGFs independently functions on cell growth stimulation. To show the direct evidence of FGF modulation by poly(P), the physical and functional interactions between poly(P) and FGFs has been examined.

*Physical Interaction Between FGF-2 and Poly(P)* - Physical interaction between FGF-2 and poly(P) was examined by a gel shift assay as described in the legend of Fig. 3. When FGF-2 was incubated with short chain [³²P]-poly(P), FGF-2 bound to the poly(P) dependent on its concentration, and poly(P) formed a complex with FGF-2 and the complex almost remained at the origin of the gel (Fig. 3). This result shows poly(P) does bind to FGF-2. Furthermore, it may possible to roughly estimate the stoichiometry between poly(P) and FGF-2. Since 8.93 pmol of FGF-2 completely trapped all [³²P]-poly(P) (16.3 pmol, estimated in terms of phosphate residue, corresponds to 0.251 pmol of poly(P) calculated from the number of average chain
length of \([^{32}P]\)-poly(P) as 65) (Fig. 3, lane 3), one molecules of poly(P) can bind to less than 35.6 molecules of FGF-2. It still be difficult to speculate the structures of poly(P)-FGF-2 complex only from this result because some FGF-2 molecules may not contribute the formation of the complex under our experimental condition. However, it may also be possible that poly(P) somehow facilitates oligomer formation of FGF-2 as in case of heparin-like glycosaminoglycans (HLGAG) (13), and a FGF-2 oligomer requires only a few phosphate molecules of poly(P) for its binding. The modulation of mitogenic activity of FGF-2 may result from the oligomer formation of FGF-2 initiated by poly(P).

*Stabilization of FGF-2 by Poly(P)* - To further examine the effect of poly(P) on FGF-2, the stability of FGF-2 with or without poly(P) was evaluated. Fig. 4A shows the degradation of FGF-2 in culture media in the presence or absence of poly(P). Intact FGF-2 clearly remained after 24 hours of incubation with poly(P) (Fig. 4A, lane 17), whereas intact FGF-2 was not observed after 24 hours of incubation without poly(P) (Fig. 4A, lane 9) or with Na-PO₄ buffer (Fig. 4A, lane 25). The half-life of FGF-2, which was calculated from the intensity of the bands of Fig. 4A was 13.7 hour when FGF-2 was incubated with poly(P), whereas it was only 4.7 hour when FGF-2 was incubated with Na-PO₄ buffer (Fig. 4B).

Since FGF-2 was physically stabilized by poly(P), stability of the biological activity of FGF-2 was also examined using BALB/c 3T3 cells. Since FGF-2
almost loses its mitogenic activity within 24 hours of incubation at 37 °C, the residual activity after preincubation (24 hours at 37 °C) of FGF-2 was examined in the presence and absence of poly(P). As shown in Fig. 4C, cells cultured in a medium containing FGF-2 that have been preincubated with poly(P) (medium #2) maintained the same population levels as that of cells cultured in a medium containing FGF-2 without preincubation (medium #3). On the other hand, a medium containing FGF-2 that had been preincubated without poly(P) (medium #4) showed slight proliferation activity but the activity decreased to the same level as that of media without FGF-2 (media #5 and #6) after 83 hours of incubation. This means that FGF-2 that had been preincubated at 37 °C was stabilized by poly(P) and maintained its proliferation activity at the same level as that of FGF-2 that had not been preincubated. In addition, the highest proliferation activity was observed in the medium containing FGF-2 and poly(P) without preincubation. This is consistent with the result shown in Fig. 2, indicating enhancement of mitogenic activity of FGF-2 by poly(P). These results indicate that poly(P) stabilizes the biological activity of FGF-2.

**Facilitation of FGF-2 Binding to Its Receptors by Poly(P)** - In order to further examine the effect of poly(P) as an FGF-2 modulator, we analyzed whether poly(P) not only stabilizes FGF-2 but facilitates FGF-2 binding to FGF receptors. The biological activity of FGF-2 is mediated by interaction with high-affinity cell surface receptors (14-16). In addition to binding to receptors, FGF-2 binds
to HSPG on the cell surface. Since many studies have indicated that the binding to HSPG facilitates FGF-2 receptor binding and activation (17-20), we removed the cell surface HSPG by sodium chlorate treatment in order to observe the direct effect of poly(P) on FGF-2 and its receptor binding (11). Sodium chlorate-treated cells were incubated with FGF-2, and the receptor-bound FGF-2 was collected and analyzed by western blotting (Fig. 5A), and intensities of the bands were quantified and plotted in Fig. 5B. The amount of FGF-2 that bound to FGF receptors in the presence of poly(P) was more than twice that in the absence of poly(P). This clearly indicates that poly(P) facilitates the binding between FGF-2 and FGF receptors. Poly(P), FGF-2, and FGF receptors may form a trimolecular complex on the cell surface. There may also be direct interactions between poly(P) and FGF receptor that facilitate FGF-2 binding and receptor dimerization as in the case of heparin (17-20).

In order to rule out the possibility that stabilization of FGF-2 by poly(P) occur during incubation with binding buffer or elution buffer, stability of FGF-2 during binding assay was evaluated. FGF-2 was incubated with poly(P) or Na-PO₄ buffer in the binding buffer or in the elution buffer at 4 °C. The amount of FGF-2 remaining in the buffer was shown in Fig. 5C. Since almost the same amount of FGF-2 was detected in buffers with poly(P) or Na-PO₄ up to 5.5 hours exposures of the binding buffer (Fig. 5C, lanes 1-4) and up to 30 min
exposures of the elution buffer (Fig. 5C, lanes 5 and 6), there is no stabilization effect of poly(P) on FGF-2 during this binding assay.

**DISCUSSION**

With regard to the mechanism of growth stimulation by poly(P), a similar effect has also been reported by adding heparin to culture media. Heparin and HLGAG are well-known potent modulators of acidic fibroblast growth factor (FGF-1) and basic fibroblast growth factor (FGF-2), and they potentiate the mitogenic activity of both FGFs (17-20). Heparin sulfate stabilizes FGFs and binds to a site on the receptor and at least one site on the growth factor. Several models propose an important role for heparin sulfate not only in facilitating FGF-2 binding to its receptor tyrosine kinase but also in promoting signaling via formation of receptor dimers. Such dimers are capable of transphosphorylation of the cytoplasmic domain of the receptor, leading to the generation of phosphotyrosine, that is an important initiator of the intracellular signaling pathway (17-20). Poly(P) may also facilitate FGF-2 binding to its receptors and promote signaling through the same binding sites of heparin sulfate, FGF-2, and its receptors. However, poly (P) and heparin have completely different chemical structures, besides both molecules are negatively charged cellular polymers. It is likely that the mechanism for modulation of FGF activity by poly(P) is different from that of heparin. Based on our results, the level of growth stimulation by poly(P) is higher than that by heparin whose
concentration is enough for maximum growth stimulation (21) (Fig. 2). This also suggests that the binding sites between poly(P) and FGFs could be different. Further analyses are needed to elucidate the detailed mechanism of interaction between poly(P) and FGF-2.

One hypothesis is that poly(P) has biological functions for controlling the activity of FGF in vivo. Since poly(P) is widely distributed in mammalian tissues (5), it is possible that degradation of FGF in tissues that have been injured is prevented if there were a mechanism for local regulation of poly(P) concentration. Furthermore, it is also possible that poly(P) interacts with other proteins or polypeptides, including growth factors, cytokines, hormones and other physiologically active factors. Recently, Kuroda et al. showed that poly(P) modulate the activity of Lon protease in E. coli depending on its nutrient condition (22). This also supports the possibility that poly(P) is a protein modulator in wide variety of organisms to regulate the functions of proteins after translation. Although there have been few reports describing the functions of poly(P) in eucaryotes, we believe that our present findings provide a key to elucidate the importance of poly(P) in higher organisms.

REFERENCES

1. Kulaev, I. S. (1979) The Biochemistry of Inorganic Polyphosphates (John Wiley & Sons, New York).
2. Wood, H. G., and Clark, J. E. (1988) *Annu. Rev. Biochem.* **57**, 235-260.

3. Kornberg, A. (1995) *J. Bacteriol.* **177**, 491-496.

4. Kulaev, I. S., Vogabov, V. M., and Kulakovskaya, T. (1999) *J. Biosci. Bioeng.* **88**, 111-129.

5. Schröder, H. C. (1999) *Progress in Molecular and Subcellular Biology* **23**, 45-81.

6. Leyhausen, G., Lorenz, B., Zhu, H., Geurtsen, W., Bohnensack, R., Muller, W. E. G., and Schröder, H. C. (1998) *J. Bone Miner. Res.* **13**, 803-812.

7. Shiba, T., JP Patent Application No. 10-242416, Aug. 28, 1998.

8. Shiba, T., US Patent No. US6,333,193B1, Dec. 25, 2001.

9. Marshall, N. J., Goodwin, C. J., and Holt, S. J. (1995) *Growth Regulation* **5**, 69-84.

10. Ahn, K., and Kornberg, A. (1990) *J. Biol. Chem.* **265**, 11734-11739.

11. Fannon, M., and Nugent, M. A. (1996) *J. Biol. Chem.* **271**, 17949-17956.

12. Souttou, B., Hamelin, R., and Crepin, M. (1994) *Cell Growth Differ.* **5**, 615-623.

13. Venkataraman, G., Sheiver, Z., Davis, J. C., Sasisekharan, R. (1999) *Proc. Natl. Acad. Sci. USA* **96**, 1892-1897.

14. Johnson, D. E., and William, L. T. (1993) *Adv. Cancer Res.* **60**, 1-41.

15. Givol, D., and Yayon, A. (1992) *FASEB J.* **6**, 3362-3369.

16. Jaye, M., Schlessinger, J., and Dionne, C. A. (1992) *Biochim. Biophys. Acta* **1135**, 185-199.

17. Kiefer, M. C., Baird, A., Nguyen, T., George-Nascimento, C., Mason, O. B., Boley, L. J., Valenzuela, P., and Barr, P. J. (1991) *Growth Factors* **5**, 115-127.
18. Kan, M., Wang, F., Xu, J, Crabb, J. W., Hou, J., and McKeehan, W. L (1993) Science 259, 1918-1921.

19. Roghani, M., Mansukhani, A., Dell’Era, P., Bellosta, C., Rifkin, D. B., and Moscatelli, D. (1994) J. Biol. Chem. 269, 3976-3984.

20. Pantoliano, N. W., Horlick, R. A., Springer, B. A., VanDyk, D. E., Tobery, T., Wetmore, D. R., Lear, J. D., Nahapetian, A. T., Bredley, J. D., and Sisk, W. P. (1994) Biochemistry 33, 10229-10248.

21. Gospodarowicz, D., and Cheng, J. (1986) J. Cell. Physiol. 128, 475-484.

22. Kuroda, A., Nomura, K., Ohtomo, R., Kato, J., Ikeda, T., Takiguchi, N., Ohtake, H., and Korngerg, A. (2001) Science 293, 705-708.

23. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Lab. Press, Plainview, New York, ed. 2).

FIGURE LEGENDS

FIG. 1. Enhancement of cell proliferation by poly(P). Cell culture and the basic proliferation assay were performed as described in the section of EXPERIMENTAL PROCEDURES. After serum starvation, NHDF (A and C) and HGF (B) cells were incubated with serum-free media, media with 1.34 mM poly(P) or media with 1.34 mM Na-PO₄ buffer. The viable cell number was quantified by the MTS method (9) in (A) and (B), and was directly counted by the use of a hematocytometer in (C). Error bars represent the standard deviations of 6 samples.
FIG. 2. **Augmentation of mitogenic activity of FGF by poly(P).** Cell culture and MTS assay (9) were performed as described in the section of EXPERIMENTAL PROCEDURE. After serum starvation, cells were incubated with serum-free D-MEM (None) or serum-free D-MEM containing Poly(P), FGF-1, Poly(P) together with FGF-1, FGF-2, Poly(P) together with FGF-2, 10% fetal bovine serum (FBS), heparin, or poly(P) together with heparin. Concentrations of Poly(P), both FGFs and heparin are 1.34 mM, 10 ng/ml and 50 µg/ml, respectively. After 30 hours of incubation at 37 °C, viable cell number was quantified by the MTS assay (9). The level of proliferation was calculated in terms of a relative value when the level of untreated cells (None) was defined as 1. Error bars represent the standard deviations of 6 samples.

FIG. 3. **Physical interaction between poly(P) and FGF-2.** Various concentrations of FGF-2 were mixed with [32P]-poly(P) (16.3 pmol in D-MEM, 10 ml reaction mixture). After the mixtures were incubated at 37 °C for 10 min, 2 µl of gel loading solution (40 % glycerol, 0.0025 % Bromophenol blue) was added and samples were applied onto 15 % polyacrylamide gel (acrylamide : bis-acrylamide = 10 : 1). After electrophoresis was performed with TBE buffer (23) at 8 V/cm², the gel was dried, and [32P]-Poly(P) and [32P]-poly(P)-FGF-2 complexes were visualized and the intensity of radioactivity was quantified by using a BAS2000 radio-image analyzer (FUJIX).

FIG. 4. **Physical and functional stabilization of FGF-2 by poly(P).** (A) Physical stabilization of FGF-2 by poly(P). FGF-2 (200 ng/ml) was incubated with D-MEM, D-MEM with 1.34 mM of poly(P) or D-MEM with 1.34 mM of Na-PO₄ buffer at 37 °C. Samples were
taken every 2 hour after 12 hours of incubation as indicated and mixed with SDS-PAGE gel loading buffer (23). Samples were separated by 14% SDS-PAGE, and FGF-2 was detected by western blotting using an anti FGF-2 antibody (Santa Cruz Biotechnology, Inc.) and goat anti-rabbit IgG alkaline phsphatase conjugate (BIO-RAD) as a second antibody. (B) Elongation of FGF-2 half-life by poly(P). The intensity of the bands that were visualized in panel A was quantified by using image analyzing software (NIH image), and plotted. (C) Functional stabilization of FGF-2 by poly(P). Cells were cultured as described in the section of EXPERIMENTAL PROCEDURES. After serum starvation, medium was replaced with 6 kinds of new serum-free D-MEM that had been prepared as follows: a medium containing 10 ng/ml of FGF-2 in combination with 1.34 mM of poly(P) (medium #1), a medium containing 10 ng/ml of FGF-2 in combination with poly(P) that had been preincubated for 24 hours at 37 °C (medium #2), a medium containing 10 ng/ml of FGF-2 (medium #3), a medium containing 10 ng/ml of FGF-2 that had been preincubated for 24 hours at 37 °C (medium #4), a medium with no preincubation and no additives (medium #5), and a medium that had been preincubated for 24 hrs at 37 °C (medium #6). After the indicated times, cell proliferation was assayed by the MTS method (9). The meanings of each symbol are summarized in the same panel. “Pre-in” means 24-hour preincubation with FGF-2. Error bars represent the standard deviations of 6 samples.

FIG. 5. Effect of poly(P) on the binding of FGF-2 to high-affinity surface receptors. (A) Visualization of FGF-2 bound to the receptor by Western blotting. Various concentrations of FGF-2 (10, 20 and 40 ng/ml) was exposed to cells with 1.34 mM of poly(P) or Na-PO₄ buffer
(control).  (B) Quantification of receptor-bound FGF-2.  The intensities of FGF-2 bands visualized in panel A were quantified by using image analyzing software (NIH image).  (C) Stability of FGF-2 during the binding assay.  FGF-2 (40 ng/ml) was incubated with poly(P) (1.34 mM) or Na-PO₄ buffer (1.34 mM) at 4 °C in the binding buffer for 2.5 or 5.5 hours, or in the elution buffer for 30 minutes.  After incubation, FGF-2 was precipitated with trichloroacetic acid and the intact protein was visualized by western blotting following the same procedure as described in EXPERIMENTAL PROCEDURES.
Relative cell growth

- Balb/c 3T3
- NHDF
- HGF
A  

|        | Na-PO₄ | Poly(P) |
|--------|--------|---------|
| 0      | 40     | 20      | 10     |
| 40     | 20     | 10      | FGF-2 (ng/ml) |

B  

FGF-2 binding (relative intensity) vs. FGF-2 concentration (ng/ml) 

Poly(P) 

Na-PO₄ 

C  

| 2.5  | 5.5  | 0.5  | Incubation (hr) |
|------|------|------|-----------------|
| Poly(P) | Na-PO₄ | Poly(P) | Na-PO₄ |
| 1    | 2    | 3    | 4    | 5    | 6    | Lanes |

FGF-2
Modulation of mitogenic activity of fibroblast growth factors by inorganic polyphosphate
Toshikazu Shiba, Daisuke Nishimura, Yumi Kawazoe, Yuichiro Onodera, Kaori Tsutsumi, Rie Nakamura and Minako Ohshiro

J. Biol. Chem. published online May 9, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M303468200

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts