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# SELECTIVE MODULATION OF THE TWO ANTAGONISTIC ACTIVITIES OF PROTEIN KINASE FA ( THE ACTIVATOR OF ATP.Mg-DEPENDENT PROTEIN PHOSPHATASE )

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The ATP.Mg-dependent protein phosphatase activating factor ( FA ) has been identified as a protein kinase. The results are unexpected since factor FA possesses two activities which are antagonistic. As a kinase, factor FA catalyzes protein phosphorylation, while as a phosphatase activator, it catalyzes protein dephosphorylation. In this report, we found that the two opposing activities of factor FA could be selectively modulated. For instance, heparin at concentrations of 0.1-0.3 mg/ml could stimulate FA to work preferentially as a kinase towards phosphorylation of proteins but simultaneously inhibit it to work as a phosphatase activator towards dephosphorylation of the same proteins. In a similar manner, alkaline pH could stimulate FA to work as a kinase but block it to work as a phosphatase activator. This is the first report providing initial evidence that the two opposing activities of factor FA can be selectively modulated in a reciprocal manner by various triggers, suggesting that a simultaneous coordinate control mechanism may well be involved in regulating the activities of factor FA in the cell.


An ATP.Mg-dependent multisubstrate/multifunctional protein phosphatase has been identified and well characterized in most mammalian nervous and nonnervous tissues ( 1-4 ). This phosphatase is inactive as isolated but can be activated in the presence of ATP.Mg and an activating factor termed FA ( 1-6 ). The activated phosphatase becomes capable of modulating the activities of diverse physiological substrates involved in the regulation of cell metabolism and functions ( 1-7 ). Only recently, factor FA has further been identified as a multisubstrate/multifunctional protein kinase ( 8-11 ). The results are unexpected since factor FA possesses two activities which are antagonistic. As a protein kinase, factor FA catalyzes protein phosphorylation, while as a protein phosphatase activator, it catalyzes protein

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Abbreviations : FA, the activating factor of ATP.Mg-dependent protein phosphatase; MBP, myelin basic protein.

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dephosphorylation. Although there is growing interest in a coordinate control of phosphorylation and dephosphorylation regulated by the selective involvement of factor FA either working preferentially as a kinase or as a phosphatase activator, however, there are no clues as to how this system is simultaneously controlled by effectors which can alter the two opposing activities in a reciprocal manner. This obviously presents an intriguing problem which deserves investigation.

In this report, we found that the two opposing activities of factor FA can be selectively modulated in a reciprocal manner by heparin and pH change, representing the first example that a simultaneous coordinate control mechanism may well be involved in regulating the two opposing activities of factor FA in the cell.

## MATERIAL AND METHODS

Material --- ATP-r-$^{32}$P was purchased from ICN. ATP, cAMP, heparin and bovine serum albumin were obtained from Sigma. Tris, MES, acetone, sucrose, dithiothreitol and Triton X-100 were from Merck. DE52, CM52 and P11 ion exchangers were from Whatman. Sepharose 4B and CNBr-activated Sepharose 4B were from Pharmacia. DEAE-Trisacryl and hydroxylapatite were from IBF. pH buffer was prepared from 200 mM MES and 200 mM Tris in a 1:1 ratio and adjusted to the desired pH with 1 N NaOH or 1 N HCl. The final concentrations of MES and Tris in the pH buffer were 50 mM. All other chemicals were of reagent grade.

Protein purification --- Phosphorylase b, phosphorylase b kinase, $^{32}$P-phosphorylase a, $^{32}$P-MBP, $^{32}$P-synapsin, factor FA, ATP.Mg-dependent protein phosphatase and central nervous system myelin basic protein ( MBP ) were purified and prepared as described in previous reports ( 1,2,5,8,12 ). $^{32}$P-MBP and $^{32}$P-synapsin were phosphorylated and prepared from ATP-r-$^{32}$P and factor FA which was identified as a MBP and synapsin kinase ( 8, 11 ). The central nervous system synapsin was purified from bovine brain as described by Ueda and Greengard ( 13 ).

Enzyme assays --- The activities of ATP.Mg-dependent protein phosphatase and factor FA were determined by methods described in previous reports ( 1, 2, 5, 8 ). Briefly, the activity of ATP.Mg-dependent protein phosphatase was typically measured after a 5-min preincubation with 0.1 mM ATP, 0.5 mM $Mg^{2+}$ ions and saturated amount of factor FA required for full activation of the inactive phosphatase. 1 mg/ml $^{32}$P-MBP or $^{32}$P-synapsin was used as the substrate. The activity of factor FA was measured by the formation of the activated ATP.Mg-dependent protein phosphatase. The assay mixture contained appropriate dilutions of factor FA, 0.1 mM ATP, 0.5 mM $Mg^{2+}$ ions and excess amount of inactive phosphatase to ensure linear activation of the phosphatase activity. A unit of protein phosphatase activity is that amount of enzyme that catalyzes the release of 1 nmol of phosphate per min. A unit of factor FA is that amount of enzyme that produces one unit of activated ATP.Mg-dependent protein phosphatase in 1-min preincubation. Unless specifically indicated, FA, as a MBP or synapsin kinase, was commonly measured in a 10-min incubation reaction mixture at 30°C containing 1 mg/ml pure MBP or synapsin, 0.5 mM EGTA, 0.1 mM [ r-$^{32}$P ] ATP ( 1 pmol of ATP = 1000 cpm ), 2 mM magnesium ions, and FA at an appropriate dilution ensuring linear rate of $^{32}$P incorporation into the substrate. The total assay volume was 0.025 ml and 0.02-ml aliquots were transferred to Whatman phosphocellulose paper, dropped into 20% ice-cold trichloroacetic acid, and then processed as described in Ref.14. A unit of FA as a kinase is that amount of enzyme that catalyzes the incorporation of 1 nmol of phosphate/min into the protein substrate.

Analytic Method --- Protein concentration was determined by the method of Lowry et al. ( 19 ) using bovine serum albumin as the standard.

## RESULTS AND DISCUSSION

The ATP.Mg-dependent protein phosphatase activating factor ( FA ) was identified as a potent and unique protein kinase capable of phosphorylating various protein substrates including central nervous system myelin basic protein ( 8 ). In sharp contrast, factor FA was also identified as a potent protein phosphatase activator to promote the dephosphorylation of central nervous system myelin basic protein ( MBP ) ( 8 ). The results are unexpected, since they catalyze reactions which are antagonistic. As a MBP kinase, factor FA catalyzes the phosphorylation of MBP, while by activating phosphatase, it catalyzes the dephosphorylation of MBP. However, as shown in Fig. 1A, heparin at concentrations of 0.1-0.3 mg/ml could dramatically stimulate factor FA to work as a MBP kinase. By contrast, when factor FA functions as a MBP phosphatase activator, heparin at the same contrations could potently inhibit it to activate the MBP phosphatase activity ( see Fig. 1B ). In order to rule out the possibility that this is simply due to the substrate-directed effect, we also tried to use central nervous system synapsin and $^{32}$P-synapsin as the testing substrates and similar results could also be obtained ( data not shown ). Taken together, the results provide initial evidence that the two antagonistic activities of factor FA can be selectively modulated in a reciprocal manner by heparin ( see Figs. 1A and 1B ). In addition to heparin as a specific modulator to selectively modulate the two opposing activities of factor FA, we found that pH change could also selectively modulate the two opposing activities of factor FA. As shown in Fig. 2A, alkaline pH appeared to favor factor FA to work as a kinase towards phosphorylation of MBP. By contrast, neutral pH seemed to favor factor FA to work as a phosphatase activator towards dephosphorylation of MBP ( see Figs. 2A and 2B ). On the other hand, alkaline pH could potently inhibit factor FA to work as a phosphatase activator ( Fig. 2B ). Similar results could also be obtained when using synapsin and $^{32}$P-synapsin as the testing substrates ( data not shown ). All the results taken together provide initial evidence that the two antagonistic activities of factor FA can be selectively modulated in a reciprocal manner by various triggers ( see Figs. 1 and 2 ).

Factor FA was first introduced as an activating factor of ATP.Mg-dependent protein phosphatase ( 1, 5, 16, 17 ). However, after many years of intensive and extensive studies, factor FA was finally recognized as an important mediator involved in the regulation of diverse physiological functions including cell metabolism, brain myelin functions, synapsin functions, microtubule assembly-disassemby, and even cell differentiation and transformation ( 1-11 ). A

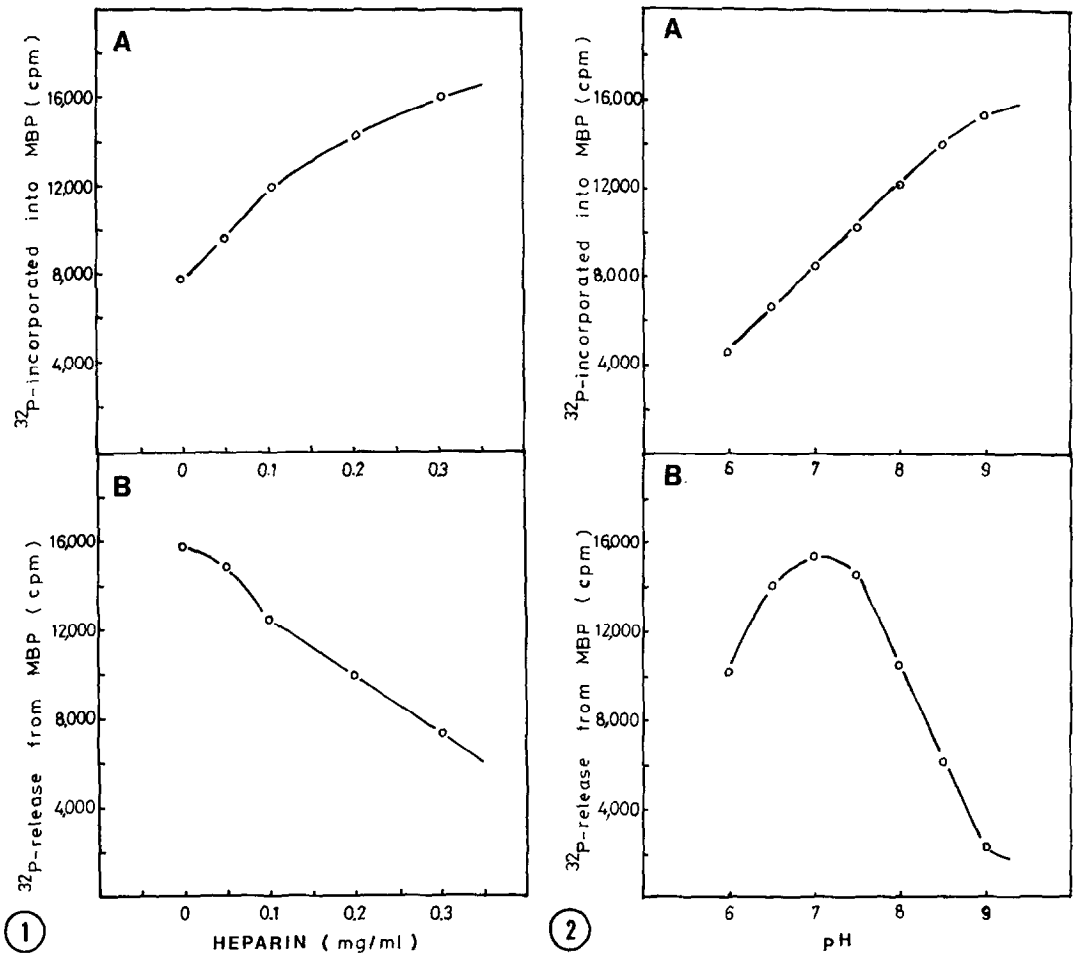


Fig. 1. Reciprocal effects of heparin on the two antagonistic activities of factor FA on the phosphorylation-dephosphorylation of MBP.

A. 1 mg/ml pure MBP was mixed with 0.1 mM ATP-r-$^{32}$P ( 1 pmol of ATP = 1000 cpm ), 2 mM $Mg^{2+}$ ions, 0.5 mM EGTA and 0.8 mU of factor FA as a kinase at 30°C for 10 min. The reaction was performed in the presence and absence of various concentrations of heparin as indicated in the 20 mM Tris buffer at pH 7.0 and started with factor FA. The total assay volumes were 0.025 ml and 0.02-ml aliquots were transfered to Whatman paper for kinase activity measurement as described in "Method". Data were taken from the average of 3 independent experiments.

B. 10 mU of inactive ATP.Mg-dependent protein phosphatase was incubated with 0.1 mM ATP, 0.5 mM $Mg^{2+}$ ions, 0.7 mU of factor FA as a phosphatase activator and various concentrations of heparin as indicated in the 20 mM Tris buffer at pH 7.0 at 30°C for 5 min. The phosphatase action was next started with 1 mg/ml $^{32}$P-MBP phosphorylated by factor FA itself. The reaction time was 5 min and assay conditions were as described in "Method". Data were taken from the average of 3 independent experiments.

Fig. 2. pH effects on the reciprocal modulation of the two antagonistic activities of factor FA on the phosphorylation-dephosphorylation of MBP.

A. 1 mg/ml pure MBP was mixed with 0.1 mM ATP-r-$^{32}$P ( 1 pmol of ATP = 1000 cpm ), 2 mM $Mg^{2+}$ ions, 0.5 mM EGTA and 0.8 mU of factor FA as a kinase at 30°C for 10 min. The reaction was performed at various pH conditions adjusted with MES/Tris pH buffer and started with factor FA. The total assay volumes were 0.025 ml and 0.02-ml aliquots were transfered to Whatman paper for kinase activity measurement as described in "Method". Data were taken from the average of 3 independent experiments.

B. 10 mU of inactive ATP.Mg-dependent protein phosphatase was incubated with 0.1 mM ATP, 0.5 mM $Mg^{2+}$ ions and 0.7 mU of factor FA as a phosphatase activator at various pH conditions as indicated at 30°C for 5 min. The phosphatase action was next started with 1 mg/ml $^{32}$P-MBP phosphorylated by factor FA itself. The reaction time was 5 min and assay conditions were as described in "Method". Data were taken from the average of 3 independent experiments.

physical association of two opposing activities in factor FA is intriguing and may represent a favorable mechanism for simultaneous regulation ( 18, 19 ). This may induce a unique form of a cyclic cascade system ( 20-22 ). Such system has been shown to possess unique features highly suited to metabolic regulation ( 20-22 ). The results as presented in this study that the two opposing activities of factor FA can be selectively modulated in a reciprocal manner by various triggers obviously provide an efficient system for a coordinate control of the phosphorylation and dephosphorylation reactions of factor FA involved in the regulation of diverse physiological functions ( 1-11 ).

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