Mannose 6-Phosphate-containing Peptides Activate Phospholipase C in Proximal Tubular Basolateral Membranes from Canine Kidney*

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To ascertain whether mannose 6-phosphate-containing peptides that bind to the insulin-like growth factor II (IGF II)/mannose 6-phosphate receptor activate phospholipase C, we determined the effect of prolifemin, transforming growth factor-β1 (TGF-β1) precursor, and β-galactosidase on production of inositol trisphosphate (Ins-P3) in basolateral membranes isolated from the renal proximal tubule of dogs. Both prolifemin and TGF-β1 precursor stimulated Ins-P3 production in a concentration-dependent manner. Maximal production was stimulated by approximately 10⁻¹⁳ M of each peptide. β-Galactosidase had no effect on Ins-P₃ generation. Neither prolifemin nor TGF-β₁ precursor potentiated IGF II-stimulated Ins-P₃ production. Mannose 6-phosphate itself had no effect on Ins-P₃ generation. However, mannose 6-phosphate-potentiated production stimulated by 10⁻¹⁴ M prolifemin or 10⁻¹¹ M TGF-β₁ precursor while inhibiting production stimulated by 10⁻¹⁴ M of either peptide. Addition of anti-mannose 6-phosphate receptor antibodies to basolateral membranes abolished prolifemin and TGF-β₁ precursor-stimulated Ins-P₃ generation. We conclude that, in addition to IGF II, mannose 6-phosphate-containing ligands for the IGF II/mannose 6-phosphate receptor activate basolateral membrane phospholipase C. Such activation could reflect a common mechanism for signal transduction by these peptides mediated via the IGF II/mannose 6-phosphate receptor.

The cation-independent mannose 6-phosphate receptor is thought to participate in translocation of newly synthesized lysosomal proteins containing a mannose 6-phosphate recognition marker to lysosomes. Approximately 80–90% of these receptors are present on intracellular membranes. The remainder are located in the plasma membrane with the mannose 6-phosphate recognition site in the extracytoplasmic region (1). The demonstration of homology between cation-independent mannose 6-phosphate receptors and receptors for insulin-like growth factor II (IGF II)¹ has suggested a dual role for this receptor as a mediator of biological responses to IGF II as well as in the targeting of lysosomal enzymes (2). We have recently shown that IGF II activates phospholipase C in isolated canine renal proximal tubular basolateral membranes (3) and that activation is enhanced by mannose 6-phosphate (4). These observations provide the first insights into the mechanism for receptor-mediated signal transduction by IGF II. In addition, they suggest a role for the extracytoplasmic mannose 6-phosphate recognition site in signal transduction.

A number of biologically active peptides or peptide precursors contain mannose 6-phosphate. These include prolifemin, a prolactin-related peptide (5), and transforming growth factor-β₁ (TGF-β₁) precursor (6). The mechanism(s) for signal transduction by either of these proteins is/are unknown. However, each has been shown to bind to the IGF II/mannose 6-phosphate receptor (5–8). To determine whether this receptor plays a role in their signal transductions, we incubated suspensions of renal basolateral membranes with prolifemin or TGF-β₁ precursor and measured production of inositol trisphosphate (Ins-P₃). Our findings suggest that, as for IGF II, signal transduction by extracellular mannose 6-phosphate-containing peptides occurs by activation of phospholipase C via the plasma membrane IGF II/mannose 6-phosphate receptor. This multifunctional receptor could serve as a transducer for cell activation by a "family" of peptides in vivo.

MATERIALS AND METHODS

Suspensions enriched for basolateral membranes originating from proximal tubular cells of dog kidney were prepared by Percoll gradient ultracentrifugation as before (3, 4, 9). Membranes were suspended in 250 mM sucrose, 100 mM NaCl, 50 mM Tris/HCl, pH 7.5, to give a protein concentration of 10 mg/ml and then frozen at ~70 °C until used in experiments.

Phospholipase C activity associated with isolated membranes was evaluated by measuring levels of Ins-P₃ in membrane suspensions as before (3, 4). Membranes (1 mg of protein) were suspended in 25 mM sucrose, 130 mM NaCl, 500 μM EGTA, 50 mM Tris, HCl, pH 7.5. The total volume was 500 μl. Sufficient CaCl₂ was included in solutions so that the concentration of free calcium was 0.2 μM. Suspensions were incubated at 30 °C for 15 s in the presence of vehicle (50 mM Tris/HCl, pH 7.5) or prolifemin (a gift of Dr. Daniel Linzer, Northwestern University, Chicago, IL, Dr. Se-Jin Lee and Dr. Daniel Nathans, Johns Hopkins University, Baltimore, MD), recombinant simian TGF-β₁ precursor disulfide-bonded complex (TGF-β₁ precursor) (Oncogen), recombinant simian TGF-β₁ (Oncogen), recombinant human IGF II (Bachem Inc., Torrance, CA), recombinant human growth hormone (Lilly), or β-galactosidase (Sigma, bovine liver G1875, 0.15 unit/mg of protein). After incubation, membranes were extracted with 1 ml of chloroform/methanol, 2:1 (v/v) containing 2.4 M HCl and re-washed with an additional 1 ml of chloroform. For some experiments, as noted, membranes were incubated for 60 min at 30 °C with 8 mg/ml rabbit anti-bovine liver cation-independent mannose 6-phosphate receptor antibodies prior to use. Ammonium sulfate-precipitated antibodies were the gift of Dr. William S. Sly (St. Louis University, St. Louis, MO).

The β-galactosidase used in experiments was assayed for mannose 6-phosphate content following hydrolysis for 2 h in 4 N H₂SO₄ (10).

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¹ The abbreviations used are: IGF II, insulin-like growth factor II; Ins-P₃, inositol trisphosphate; TGF-β₁, transforming growth factor-β₁; HPLC, high pressure liquid chromatography; EGTA, ethylenediamine(N,N'-diacetic acid)}
It was found to contain 54 μg of mannose 6-phosphate/mg of protein. Additional evidence consistent with the presence of mannose 6-phosphate in the β-galactosidase was obtained by measuring binding of 125I-IGF II to basolateral membranes in the absence and presence of β-galactosidase using methodology described previously (11). As in the studies of Kiess et al. (12) binding of 125I-IGF II was inhibited by β-galactosidase. Binding of 125I-IGF II tracer in the presence of β-galactosidase was divided by binding in the absence of β-galactosidase (B/Bo) was 0.67 ± 0.1, 0.55 ± 0.04, 0.35 ± 0.08, 0.20 ± 0.06, and 0.11 ± 0.02 in the presence of 10⁻¹², 10⁻¹⁰, 10⁻⁸, 10⁻⁶, and 10⁻⁴ M β-galactosidase, respectively (u = 3 experiments).

To measure Ins-P₃ originating from membranes, water-soluble inositol phosphates contained in the methanol layer of the extraction mixture were separated by HPLC using a Mono Q HR 5/5 anion exchange column (Pharmacia LKB Biotechnology, Inc.) exactly as detailed before (3, 4). After HPLC separation, the Ins-P₃ peak material was desalted and dephosphorylated, and free myo-inositol was measured using an enzymatic fluorometric assay (3, 4, 13).

Metabolism of Ins-P₃ in basolateral membrane suspensions was measured as before (3, 4) by comparing radioactivities in the Ins-P₃-containing HPLC fractions prior to incubation and after incubation of basolateral membranes with approximately 2 nCi of [3H]inositol 1,4,5-trisphosphate ([3H]Ins-P₃) (Du Pont-New England Nuclear, 3.6 Ci/mmol).

Protein was measured by the method of Lowry et al. (14). Dunnnett's multiple comparison procedure (15) was used for statistical analysis under the condition that the changes in levels of Ins-P₃ illustrated in Figs. 1 and 2 reflect inhibition of Ins-P₃ breakdown by prolactin and/or TGF-β₁ precursor. To determine whether this is the case, we measured dephosphorylation of [3H]Ins-P₃ added to membranes at time 0 in the absence or presence of 10⁻¹⁴ M of each peptide. During 15 s of incubation 8.0 ± 2.2% of [3H]Ins-P₃ added to membranes in the absence of peptide was dephosphorylated. The extent of dephosphorylation was not significantly different in the presence of prolactin or TGF-β₁ precursor (Table I). Thus the effect of neither mannose 6-phosphate-containing peptide can be explained by inhibition of Ins-P₃ breakdown.

We have shown previously that millimolar concentrations of mannose 6-phosphate potentiate IGF II-stimulated Ins-P₃ production in basolateral membranes, despite mannose 6-phosphate itself having no effect on production or breakdown. Potentiation was observed using 10⁻¹⁰, 10⁻⁹, and 10⁻⁸ M IGF II (4). We postulated that this action could reflect a role for the mannose 6-phosphate moiety as a regulator of IGF II signal transmission and suggested that the effect of free mannose 6-phosphate in vitro might be duplicated by far smaller concentrations of mannose 6-phosphate linked to an appropriate peptide modulator (4). To ascertain whether either prolactin or TGF-β₁ precursor is such a modulator, we determined the effect of prolactin or TGF-β₁ precursor on IGF II activation of phospholipase C. To this end, we measured Ins-P₃ production in basolateral membranes incubated with 10⁻⁹ M IGF II, the concentration of IGF II at which binding is half-maximal (11), and either no additional peptide, or prolactin or TGF-β₁ at concentrations of 10⁻¹⁰, 10⁻⁹, and 10⁻⁸ M.

**RESULTS**

To ascertain whether mannose 6-phosphate-containing peptides activate phospholipase C in isolated basolateral membranes, we first measured levels of Ins-P₃ in suspensions that were incubated in the absence of peptide or in the presence of various concentrations of prolactin, TGF-β₁ precursor, or the mannose 6-phosphate-containing lysosomal enzyme β-galactosidase for 15 s prior to extraction. Inclusion of prolactin (Fig. 1) or TGF-β₁ precursor (Fig. 2) in incubations resulted in a concentration-dependent increase of Ins-P₃ in suspensions. Significantly increased levels (p < 0.05, Dunnett's multiple comparison procedure) compared with those measured in the absence of peptide were detected in suspensions incubated with 10⁻¹⁴ M and higher concentrations of prolactin or TGF-β₁ precursor. Maximal increases were observed at approximately 10⁻¹³ M of either peptide. In contrast, no concentration of β-galactosidase (10⁻¹³-10⁻⁴ M) significantly stimulated Ins-P₃ production (Fig. 3). Therefore, some (prolactin, TGF-β₁ precursor) but not all (β-galactosidase) mannose 6-phosphate-containing peptides activate phospholipase C in suspensions of basolateral membranes.

Levels of Ins-P₃ in membrane suspensions reflect breakdown as well as production of Ins-P₃ (3, 4). It is possible that the changes in levels of Ins-P₃ illustrated in Figs. 1 and 2 reflect inhibition of Ins-P₃ breakdown by prolactin and/or TGF-β₁ precursor. To determine whether this is the case, we measured dephosphorylation of [3H]Ins-P₃ added to membranes at time 0 in the absence or presence of 10⁻¹⁴ M of each peptide. During 15 s of incubation 8.0 ± 2.2% of [3H]Ins-P₃ added to membranes in the absence of peptide was dephosphorylated. The extent of dephosphorylation was not significantly different in the presence of prolactin or TGF-β₁ precursor (Table I). Thus the effect of neither mannose 6-phosphate-containing peptide can be explained by inhibition of Ins-P₃ breakdown.

We have shown previously that millimolar concentrations of mannose 6-phosphate potentiate IGF II-stimulated Ins-P₃ production in basolateral membranes, despite mannose 6-phosphate itself having no effect on production or breakdown. Potentiation was observed using 10⁻¹⁰, 10⁻⁹, and 10⁻⁸ M IGF II (4). We postulated that this action could reflect a role for the mannose 6-phosphate moiety as a regulator of IGF II signal transmission and suggested that the effect of free mannose 6-phosphate in vitro might be duplicated by far smaller concentrations of mannose 6-phosphate linked to an appropriate peptide modulator (4). To ascertain whether either prolactin or TGF-β₁ precursor is such a modulator, we determined the effect of prolactin or TGF-β₁ precursor on IGF II activation of phospholipase C. To this end, we measured Ins-P₃ production in basolateral membranes incubated with 10⁻⁹ M IGF II, the concentration of IGF II at which binding is half-maximal (11), and either no additional peptide, or prolactin or TGF-β₁ at concentrations of 10⁻¹⁰, 10⁻⁹, and 10⁻⁸ M.

**TABLE I**

<table>
<thead>
<tr>
<th>10⁻¹⁴ M peptide</th>
<th>[3H]Ins-P₃ breakdown</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>8.0 ± 2.2</td>
</tr>
<tr>
<td>Prolactin</td>
<td>17.0 ± 5.4</td>
</tr>
<tr>
<td>TGF-β₁ precursor</td>
<td>19.7 ± 0.8</td>
</tr>
<tr>
<td>TGF-β₁</td>
<td>16.2 ± 4.0</td>
</tr>
</tbody>
</table>
TGF-βl precursor in the presence of IGF II was not significantly different from the increase stimulated by TGF-β1 precursor in the absence of IGF II. These findings are not consistent with a role for either proliferin or TGF-βl precursor as a potentiator of IGF II-mediated phospholipase C activation.

TGF-β is known to stimulate Ins-P₃ production in rat-1 fibroblasts (16). To ascertain whether the action of TGF-β1 precursor to generate Ins-P₃ in isolated basolateral membranes results from TGF-β1 that may have been present as a contaminant of the precursor, we determined the effect of TGF-β1 on Ins-P₃ production. TGF-β1 stimulated Ins-P₃ production in basolateral membranes in a concentration-dependent manner (Fig. 4). Therefore, the mature peptide, if present as a contaminant of TGF-β1 precursor, could be causative of phospholipase C activation. However, the data shown in Figs. 2 and 4 and Table I demonstrate that this is not the case. Maximal stimulation of Ins-P₃ production by TGF-β1 in basolateral membranes is greater than that by the precursor, and maximal stimulation occurs at a higher concentration of TGF-β1 (compare Figs. 2 and 4). Since dephosphorylation of [³H]Ins-P₃ is not significantly different in the absence or presence of 10⁻¹⁴ M TGF-β1 precursor (Table I), the findings in Figs. 2 and 4 cannot be explained by inhibition of Ins-P₃ breakdown by TGF-β1 but rather reflect differences in phospholipase C activation. If the action of TGF-β1 precursor resulted from contaminating TGF-β1, peak stimulation by the contaminated precursor should be of approximately the same magnitude as that by TGF-β1 and should occur at a concentration equal or higher, but not lower, than that at which the action of 10⁻¹⁴ M TGF-β1 is maximal. Therefore, the effect of the precursor cannot be explained by contaminating TGF-β1.

Both proliferin and TGF-β1 precursor are known to bind to the IGF II/mannose 6-phosphate receptor (5-8). To provide insight as to whether activation of phospholipase C by one or both peptides is mediated via this receptor we determined whether mannose 6-phosphate affects this process. As was the case for activation of phospholipase C by IGF II (4), mannose 6-phosphate enhanced activation by both 10⁻¹⁴ M proliferin and 0 or varying concentrations of mannose 6-phosphate. Data represent mean ± S.E. of five experiments.

**TABLE II**

Levels of Ins-P₃ in basolateral membrane suspensions

Levels in Ins-P₃ in basolateral membrane suspensions were measured as described in the text. Data represent mean ± S.E. Paired incubations were carried out in the absence or presence of 10⁻⁴ M IGF II with or without varying concentrations of proliferin or TGF-β1 precursor.

<table>
<thead>
<tr>
<th>Concentration (M)</th>
<th>Ins-P₃ (n = 3)</th>
<th>TGF-β1 precursor</th>
<th>Ins-P₃ (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No IGF II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.62 ± 0.05</td>
<td></td>
<td>0.88 ± 0.05</td>
</tr>
<tr>
<td>10⁻¹⁴</td>
<td>1.31 ± 0.16</td>
<td>10⁻¹⁴</td>
<td>1.07 ± 0.04</td>
</tr>
<tr>
<td>10⁻¹⁰</td>
<td>2.12 ± 0.34</td>
<td>10⁻¹⁰</td>
<td>1.48 ± 0.14</td>
</tr>
<tr>
<td>10⁻¹¹</td>
<td>2.23 ± 0.33</td>
<td>10⁻¹¹</td>
<td>1.30 ± 0.12</td>
</tr>
<tr>
<td>10⁻¹⁰</td>
<td>2.36 ± 0.53</td>
<td>10⁻¹⁰</td>
<td>1.54 ± 0.18</td>
</tr>
<tr>
<td>10⁻⁹</td>
<td>2.34 ± 0.30</td>
<td>10⁻⁹</td>
<td>1.31 ± 0.16</td>
</tr>
<tr>
<td>10⁻⁸</td>
<td>2.33 ± 0.41</td>
<td>10⁻⁸</td>
<td>1.22 ± 0.10</td>
</tr>
<tr>
<td>10⁻⁷</td>
<td>2.45 ± 0.63</td>
<td>10⁻⁷</td>
<td>1.19 ± 0.08</td>
</tr>
<tr>
<td>10⁻⁶</td>
<td>2.31 ± 0.42</td>
<td>10⁻⁶</td>
<td>1.44 ± 0.19</td>
</tr>
</tbody>
</table>

**FIG. 4.** Levels of Ins-P₃ in basolateral membrane suspensions in the absence (○) or presence (○) of varying concentrations of TGF-β1. Data represent mean ± S.E. of three experiments.

**FIG. 5.** Levels of Ins-P₃ in basolateral membrane suspensions in the absence (○) or presence (○) of 10⁻¹⁴ M proliferin and 0 or varying concentrations of mannose 6-phosphate. Data represent mean ± S.E. of four experiments.

**FIG. 6.** Levels of Ins-P₃ in basolateral membrane suspensions in the absence (○) or presence (○) of 10⁻¹⁴ M TGF-β1 precursor and 0 or varying concentrations of mannose 6-phosphate. Data represent mean ± S.E. of five experiments.
proliferin or TGF-β1 precursor (Table III).

In contrast to the action of mannose 6-phosphate to enhance the activation of phospholipase C by TGF-β1 precursor, there was no effect of this hexose phosphate on activation of phospholipase C by TGF-β1 (Table III). This observation provides strong evidence that TGF-β1 acts via a receptor other than the IGF II/mannose 6-phosphate receptor.

Mannose 6-phosphate has been shown to inhibit binding of both proliferin (5) and TGF-β1 precursor (6) to the IGF II/mannose 6-phosphate receptor. It would be expected that 10 mM mannose 6-phosphate should reduce binding by 75-95% (5, 6). This magnitude of reduction would not be sufficient to decrease activation of phospholipase C by 10^{-11} M proliferin or TGF-β1 precursor (Figs. 5 and 6), because maximal activation in the absence of mannose 6-phosphate occurs at 10^{-13} M peptide (Figs. 1 and 2). However, such a reduction in binding might be sufficient to decrease activation by 10^{-14} M proliferin or TGF-β1 precursor because the magnitude of Ins-P_3 generation is concentration-dependent at 10^{-14} M peptide (Figs. 1 and 2). To determine whether decreased activation is observed under these conditions, we measured Ins-P_3 production in basolateral membrane suspensions incubated with or without 10^{-14} M proliferin or TGF-β1 precursor in the presence and absence of 10 mM mannose 6-phosphate or mannose 1-phosphate. As shown in Table IV, 10 mM mannose 6-phosphate inhibited production of Ins-P_3 in the presence of both 10^{-14} M proliferin and TGF-β1 precursor. In contrast, 10 mM mannose 1-phosphate had no such effect. These observations establish that activation of phospholipase C at lower concentrations of proliferin and TGF-β1 precursor is inhibited by 10 mM mannose 6-phosphate. The data suggest that both peptides activate phospholipase C via an interaction with the IGF II/mannose 6-phosphate receptor.

To address the question of whether proliferin and TGF-β1 activate phospholipase C through the IGF II/mannose 6-phosphate receptor using other methodology, we incubated basolateral membranes with anti mannose 6-phosphate receptor antibodies prior to addition of vehicle or peptide. These antibodies have been shown to inhibit endocytosis of β-glucuronidase and recycling of IGF II/mannose 6-phosphate receptors in human fibroblasts (17). Incubation with antibodies did not affect Ins-P_3 production stimulated by 10^{-10} M human growth hormone. Therefore, the antibodies do not inhibit phospholipase C per se. However, 10^{-10} M proliferin nor 10^{-14} M TGF-β1 precursor stimulated Ins-P_3 generation following incubation with antibody (Table V). These findings provide strong evidence that proliferin and TGF-β1 precursor activate phospholipase C via the IGF II/mannose 6-phosphate receptor.

### Table III

**Levels of Ins-P_3 in basolateral membrane suspensions**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>No hexose</th>
<th>Mannose 6-phosphate</th>
<th>Mannose 1-phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>No peptide</td>
<td>0.64 ± 0.03</td>
<td>0.74 ± 0.08</td>
<td>0.69 ± 0.07</td>
</tr>
<tr>
<td>Proliferin</td>
<td>1.49 ± 0.01</td>
<td>3.10 ± 0.25</td>
<td>1.60 ± 0.04</td>
</tr>
<tr>
<td>TGF-β1 precursor</td>
<td>1.67 ± 0.09</td>
<td>2.35 ± 0.13</td>
<td>1.64 ± 0.03</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>2.22 ± 0.17</td>
<td>2.16 ± 0.13</td>
<td></td>
</tr>
</tbody>
</table>

### Table IV

**Levels of Ins-P_3 in basolateral membrane suspensions**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>No hexose</th>
<th>Mannose 6-phosphate</th>
<th>Mannose 1-phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>No peptide</td>
<td>0.74 ± 0.04</td>
<td>0.73 ± 0.07</td>
<td>0.69 ± 0.07</td>
</tr>
<tr>
<td>Proliferin</td>
<td>1.33 ± 0.02</td>
<td>0.79 ± 0.04</td>
<td>1.37 ± 0.06</td>
</tr>
<tr>
<td>TGF-β1 precursor</td>
<td>1.19 ± 0.04</td>
<td>0.75 ± 0.09</td>
<td>1.25 ± 0.03</td>
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</table>

### Table V

**Levels of Ins-P_3 in basolateral membrane suspensions**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>No antibody</th>
<th>Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol/mg protein/15 s</td>
<td></td>
</tr>
<tr>
<td>No peptide</td>
<td>0.60 ± 0.04</td>
<td>0.60 ± 0.04</td>
</tr>
<tr>
<td>Growth hormone</td>
<td>1.34 ± 0.26</td>
<td>1.34 ± 0.26</td>
</tr>
<tr>
<td>Proliferin</td>
<td>0.73 ± 0.05</td>
<td>0.73 ± 0.05</td>
</tr>
<tr>
<td>TGF-β1 precursor</td>
<td>0.87 ± 0.03</td>
<td>0.87 ± 0.03</td>
</tr>
</tbody>
</table>

**Discussion**

We have demonstrated previously that IGF II activates phospholipase C in basolateral membranes isolated from the canine renal proximal tubule (9). Presently, we have shown that proliferin and TGF-β1 precursor, two mannose 6-phosphate-containing peptides that bind to the IGF II/mannose 6-phosphate receptor (5-8), act similarly. The physiological actions of neither proliferin nor TGF-β1 precursor are well defined. The former peptide is a 224-amino acid prolactin-related glycoprotein secreted by a number of mouse cell lines during growth and by mouse placenta in mid- and late gestation. It circulates at a concentration of approximately 10^{-7} M in pregnant mice and is present at somewhat lower concentrations in amnionic fluid (18). Proliferin is closely related, if not identical, to mitogen-related protein (19). It is thought that proliferin is an autocrine growth factor for cells in culture and a placental hormone that regulates growth and/or differentiation of specific cells in the mouse fetus or maternal tissues (5, 18). TGF-β1 precursor is a 390-amino acid glycoprotein from which the 112-amino acid chain of mature TGF-β1 is derived (20, 21). TGF-β1 is known to exert growth-stimulating and -inhibiting actions on a variety of cell types (22). It is not known with certainty whether TGF-β1 precursor has independent effects. The precursor was shown to inhibit growth of mink lung epithelial cells at a concentration as low as 10^{-11} M, but it was 50 fold less active than TGF-β1 itself. However, the inhibition curve of the precursor had a slightly altered slope when compared with the mature growth factor. Therefore it is suggested that the precursor itself may be biologically active (20). Latent TGF-β1 is a high molecular mass form of TGF-β1 secreted by platelets (20). It blocks the binding of TGF-β1 precursor to isolated IGF II/mannose 6-
phospholipase C by both proligerin and TGF-β1 precursor (Figs. 5 and 6). However, whereas millimolar quantities are required to potentiate the precursor are stimulated by micromolar concentrations of binding cannot explain the effect of mannose 6-phosphate to determine in our studies. Mannose 6-phosphate itself has no potentiate proliferin or TGF-β1 precursor activation of phospholipase C in basolateral membranes with another action of proliferin in vitro. Growth hormone also stimulates phospholipase C in basolateral membranes, half-maximally at between 10⁻¹⁰ and 10⁻⁹ M peptide. Prolactin has no such effect (26). Although proliferin has a closer chemical homology to mouse prolactin than to mouse growth hormone (18), it is possible that the action of proliferin in basolateral membranes occurs via the growth hormone receptor. However, this is rendered unlikely by the fact that its half-maximal effect on phospholipase C activation occurs at a concentration 4 orders of magnitude lower than either human or bovine growth hormone (26) and by the observation that proliferin binds to neither growth hormone nor prolactin receptors in mouse (5). The action of subpicomolar concentrations of TGF-β1 precursor and TGF-β1 to activate phospholipase C is consistent with effects described in other systems in vitro (20) including isolated kidney cells (27).

As is the case for IGF II (4), mannose 6-phosphate potentiates the activation of phospholipase C by both 10⁻¹¹ M proligerin and TGF-β1 precursor (Figs. 5 and 6). However, whereas millimolar quantities are required to potentiate the IGF II effect (4), the actions of both proliferin and TGF-β1 precursor are stimulated by micromolar concentrations of mannose 6-phosphate. The mechanism by which mannose 6-phosphate potentiates activation of phospholipase C by ligands of the IGF II/mannose 6-phosphate receptor is not determined in our studies. Mannose 6-phosphate itself has no effect on phospholipase C. Therefore potentiation cannot result from an additive effect of ligand plus hexose phosphate. In the case of IGF II, potentiation could conceivably result from changes in ligand binding, since mannose 6-phosphate is known to enhance binding of IGF II to the receptor, possibly by displacing endogenous inhibitory ligands (28). However, we previously have shown that this cannot explain potentiation in basolateral membranes (4). Furthermore, enhanced binding cannot explain the effect of mannose 6-phosphate to potentiate proliferin or TGF-β1 precursor activation of phospholipase C. This is because mannose 6-phosphate reduces binding of these peptides to the IGF II/mannose 6-phosphate receptor (5, 6). In fact, 10 mM mannose 6-phosphate inhibits activation of phospholipase C by 10⁻¹⁴ M proligerin or TGF-β1 precursor (Table IV). The apparent decrease in potentiation of TGF-β1 precursor-induced phospholipase C activation by 10⁻⁴ M mannose 6-phosphate compared with 10⁻¹⁴ M mannose 6-phosphate (Fig. 6) could reflect inhibition of precursor binding. The findings illustrated in Fig. 6 and Table IV demonstrate that millimolar quantities of mannose 6-phosphate regulate peptide-stimulated phospholipase C activation in more than one way. They suggest that at low proliferin or TGF-β1 precursor concentrations reduction in peptide binding by high concentrations of mannose 6-phosphate can overcome its action to enhance Ins-P₃ generation.

It is possible that potentiation of phospholipase C activation reflects an action of free mannose 6-phosphate bound to the receptor to modulate signal transduction by a peptide ligand. It is known that IGF II and mannose 6-phosphate bind to separate sites on the IGF II/mannose 6-phosphate receptor (29) and that the receptor has two mannose 6-phosphate binding sites/polypeptide chain (30). The binding of mannose 6-phosphate to one of the sites could potentiate activation of phospholipase C by either IGF II, proliferin, or TGF-β1 precursor bound to one of the other sites. Whether such interactions could occur in vivo is unknown.

The primary structures of proliferin (31) and TGF-β1 precursor (32) are dissimilar. Therefore, it is unlikely that recognition of these peptides by the IGF II/mannose 6-phosphate receptor depends upon structural similarities other than the presence of mannose 6-phosphate itself. Accordingly, other proteins that contain mannose 6-phosphate may also activate phospholipase C. It has been shown recently that chicken and frog mannose 6-phosphate receptors fail to bind IGF II (33, 34). This has prompted speculation that the emergence of a high affinity IGF II binding site on the receptor occurred in evolution following the divergence of mammals from other vertebrates (34). It is possible that activation of phospholipase C by proliferin and TGF-β1 precursor in basolateral membranes reflects a mechanism for signal transduction by mannose 6-phosphate-containing peptides that predates the effect of IGF II. It should be noted that mannose 6-phosphate content and ability to bind to the IGF II/mannose 6-phosphate receptor are not in themselves sufficient for activation of phospholipase C, since β-galactosidase, a known ligand (12), has no such action (Fig. 3). This is not surprising in view of the absence of data implicating β-galactosidase in trans-membrane signaling.

It is possible that both proliferin and TGF-β1 precursor activate proximal tubular phospholipase C in vivo. Alternatively, their effects on isolated basolateral membranes could reflect the actions of other mannose 6-phosphate-containing peptides. Such ligands for the IGF II/mannose 6-phosphate receptor need not be restricted to circulating or locally produced growth factors. Membrane-associated proteins such as the epidermal growth factor receptor that contains mannose 6-phosphate may be one component of an "growth-regulating networks" based on protein-carbohydrate interactions and involving growth factors and their receptors. She postulates that the IGF II/mannose 6-phosphate receptor, by virtue of its binding sites for IGF II and mannose 6-phosphate, may be one component of an interacting network of growth-regulating molecules and receptors (36). It is possible that signal transduction by a large "family" of peptides with dissimilar structures is mediated or modulated through this multifunctional hormone/mannose 6-phosphate receptor.

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Mannose 6-Phosphate Peptides Activate Phospholipase C


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