

# Phosphorylation activates the insulin receptor tyrosine protein kinase

(histone/phosphoproteins/insulin receptor subunit/autophosphorylation/dephosphorylation)

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**ABSTRACT** Preparations of insulin receptor from cultured 3T3-L1 adipocytes and human placenta previously was found to catalyze the phosphorylation of the 90,000-dalton component of the insulin receptor on tyrosine residues. This insulin-dependent phosphorylation has now been shown to coincide with the generation of an activated, insulin-independent, receptor protein kinase. Activation is dependent upon ATP, divalent cations ( $Mg^{2+}$  and  $Mn^{2+}$ ), and insulin (half-maximal activation occurs at 6–8 nM insulin). The time required for activation is consistent with that needed for insulin-dependent self-phosphorylation of the receptor present in eluates from wheat germ lectin-agarose columns and in preparations of affinity-purified placental receptor. Activation proceeds unabated in the presence of soybean trypsin inhibitor at 0.1 mg/ml and the activated, insulin-independent, protein kinase sediments in 5–20% sucrose gradients at the same position as the unmodified receptor. Under steady-state conditions, the phosphorylated receptor binds insulin in the same fashion as the unmodified receptor. It is proposed that the self-phosphorylated form of the receptor is the insulin-activated protein kinase that catalyzes the phosphorylation of exogenous protein and peptide substrates. A corollary of this hypothesis is that enzymatic dephosphorylation may be essential for reversibly terminating the activity of the insulin-receptor protein kinase.

Insulin activates a protein kinase that catalyzes the phosphorylation of the insulin receptor in intact cells (1–3) and in preparations of purified insulin receptor (4). *In vitro*, the insulin receptor derived from cultured 3T3-L1 adipocytes and human placenta is phosphorylated on tyrosine residues in the 90,000-dalton subunit of the receptor (3, 5). Evidence that insulin-dependent tyrosine kinase activity copurifies with the receptor to near-homogeneity suggests that the receptor itself is the protein kinase. However, it is not known which of its subunits possesses this activity. All of the tyrosine protein kinases that have been described thus far undergo self-phosphorylation. This modification, however, has not been reported to affect enzyme function.

We have now found that incubation of the insulin receptor with insulin, ATP, and divalent cations under conditions in which it becomes phosphorylated stimulates its subsequent ability to catalyze the phosphorylation of exogenous substrates. The phosphorylated receptor remains fully active after gel filtration at pH 6.0, a procedure which restores insulin dependency to the insulin-treated, unphosphorylated receptor.

## MATERIALS AND METHODS

**Materials.** To prepare insulin receptor, cultured 3T3-L1 adipocytes were grown and induced to differentiate as described (6). A glycoprotein-rich fraction derived from solubilized mem-

branes of either human placenta or 3T3-L1 adipocytes was prepared by wheat germ agglutinin-agarose chromatography as described (3). This procedure purified the insulin receptor approximately 15-fold from the solubilized membranes and contained 0.3–1.3 mg of protein and 1–2 pmol of insulin binding capacity per ml. It was used either directly (with or without dialysis to remove *N*-acetylglucosamine) or after storage at  $-20^{\circ}C$ . Unless otherwise indicated, the preparations of insulin receptor used for these studies were eluates from wheat germ agglutinin-agarose. Preparations from placenta and adipocytes behaved similarly in all of the studies reported. For some experiments, the placental insulin receptor was further purified by affinity chromatography on insulin-sepharose. Purification was performed as reported (7) with the following modifications: (i) the wheat germ lectin purification described above was used instead of DEAE-cellulose chromatography; (ii) the receptor was eluted from insulin-Sepharose with 3.0 M urea at pH 7.4 and was immediately adsorbed to, washed, and eluted from a second wheat germ agglutinin-agarose column. This eluate, purified 400-fold from the solubilized membranes, bound 90 pmol of insulin per mg of protein.

$[\gamma\text{-}^{32}P]ATP$  (3,000 Ci/mmol; 1 Ci =  $3.7 \times 10^{10}$  Bq) was from Amersham, insulin was from Eli Lilly, histone H2B and bacterial alkaline phosphatase were from Worthington, and angiotensin II was from Sigma. Agarose-bound wheat germ agglutinin was from Vector (Burlingame, CA) and Liquiscint was from National Diagnostics (Somerville, NJ).

**General Methods.** Insulin-binding activity was measured as described in ref. 7, and protein was determined by the method of Bensadoun and Weinstein (8).

**Protein Kinase Assays.** Assays for tyrosine protein kinase activity were performed in a final volume of 50–60  $\mu$ l containing 0.03–0.05% Triton X-100, 20 mM HEPES buffer at pH 7.4, 10% (vol/vol) glycerol, 30 mM NaCl, 12 mM  $MgCl_2$ , 2 mM  $MnCl_2$ , and 10  $\mu$ g of bovine serum albumin per ml. Reactions were initiated by the addition of  $[\gamma\text{-}^{32}P]ATP$  (final concentration, 20–25  $\mu$ M; 15–45 cpm/fmol) and were at  $23^{\circ}C$ . Under standard conditions, the amount of receptor used in the kinase assay was sufficient to bind 10–15 fmol of insulin. Where indicated, histone was present at 0.4 mg/ml. Reactions were terminated and the products of the reaction were resolved by three methods.

(i) NaDodSO<sub>4</sub>/polyacrylamide gel electrophoresis followed by excision of  $^{32}P$ -labeled protein bands as described (3). Gels were treated for 1 hr at  $55^{\circ}C$  in 1 M NaOH prior to drying, radioautography, and band excision in order to remove any serine phosphate incorporated by impurities in the receptor preparation (ref. 9; unpublished data). This procedure removed all of the  $^{32}P$  incorporated into serine residues of histone by purified cAMP-dependent protein kinase and no more than 10% of the  $^{32}P$  incorporated into tyrosine residues on histone by the

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Abbreviation: p[NH]ppA, adenosine 5'-[ $\beta$ ,  $\gamma$ -imido]triphosphate.

purified placental insulin receptor (3).

(ii) The phosphocellulose paper assay described by Casnellie *et al.* (10) and Glass *et al.* (11) was used for the studies with-angiotensin.

(iii) Precipitation was carried out with 10% trichloroacetic acid (in the presence of bovine serum albumin at 1 mg/ml), and the precipitated protein was collected by centrifugation, re-suspended in 0.25 ml of 0.5 M NaOH, and heated for 60 min at 55°C. The solutions were then neutralized with HCl, reprecipitated with 10% trichloroacetic acid, collected on glass fiber filters, and assayed in Liquiscint.

**Sucrose Gradients.** Centrifugation of 0.3 ml of wheat germ agglutinin-agarose eluate was performed in an SW 41 rotor at  $286,000 \times g$  for 20 hr at 4°C in 5–20% sucrose gradients in 20 mM Hepes, pH 7.4/0.1% Triton X-100/0.1% bovine serum albumin. Fractions were then assayed for insulin binding activity (100  $\mu$ l) and alkali-resistant histone kinase activity (25  $\mu$ l) as described above. The insulin binding activity was recovered in fractions 13–15 (numbered from the bottom of the gradient) in the presence of 0.1 M NaCl and in fractions 16–18 in the presence of 0.5 M NaCl. In both cases, with unmodified receptor, all of the histone tyrosine kinase activity was insulin-stimulated and cosedimented with a single peak of insulin-binding activity.

**Minicolumn Gel Filtration.** Enzyme that had been preincubated under various conditions at pH 7.4 was adjusted to pH 6.0 with 1 M Hepes pH 6.0 buffer and incubated for 2 min at 23°C. The material (100  $\mu$ l) was then applied to 1-ml Sephadex G-75 columns previously equilibrated with 20 mM Hepes, pH 6.0/0.1% Triton X-100/0.1 M NaCl. Fractions were collected at 23°C by spinning the columns at low speed in a table-top centrifuge for 30 sec after each application of 50 or 60  $\mu$ l of the same buffer. Blue dextran, insulin-binding activity, and  $^{32}$ P-labeled phosphoreceptor eluted in fractions 3–5; ATP and free insulin eluted after fraction 6. Fractions were assayed at pH 7.4 for protein kinase activity in the standard reaction mixture. Recovery of histone kinase activity from each column was approximately 40–50% under all experimental conditions.

## RESULTS

Preincubation of the insulin receptor at pH 7.4 with 12 mM  $Mg^{2+}$ , 2 mM  $Mn^{2+}$ , and insulin at 1  $\mu$ g/ml for 15 min followed by the addition of 20  $\mu$ M ATP for 6 min stimulated the initial rate of histone phosphorylation by the enzyme (Fig. 1). Preparations of receptor so treated catalyzed the phosphorylation of tyrosine residues on histone at a constant rate and did not show the initial lag exhibited by enzyme that had been incubated with insulin but exposed to both ATP and histone at the start of the reaction (Fig. 1). Neither the addition of 20  $\mu$ M adenosine 5'-[ $\beta$ ,  $\gamma$ -imido]triphosphate (p[NH]ppA) nor the addition of 100  $\mu$ M GTP, UTP, ADP, or AMP activated the histone kinase activity when substituted for ATP during the preincubation. Activation by ATP was dependent upon the presence of  $Mg^{2+}$  (12 mM) and  $Mn^{2+}$  (2 mM) and did not occur when the reaction was performed with 10 mM EDTA and no added divalent cations. With angiotensin as substrate for the tyrosine phosphorylation reaction (12, 13), the phosphorylated receptor exhibited a 3- to 4-fold increase in  $V_{max}$  (Fig. 2); the  $K_m$  for angiotensin remained unchanged. Activation of the enzyme by ATP was insulin-dependent (Fig. 3). The concentration of insulin required for half-maximal receptor phosphorylation and half-maximal activation of histone tyrosine kinase activity was approximately 6–8 nM. The ability of the receptor to bind  $^{125}$ I-labeled insulin (0.17–1.7 nM) after a 1-hr incubation at 23°C (7) was not affected by the presence of ATP,  $Mg^{2+}$ , and  $Mn^{2+}$  during the binding assay.

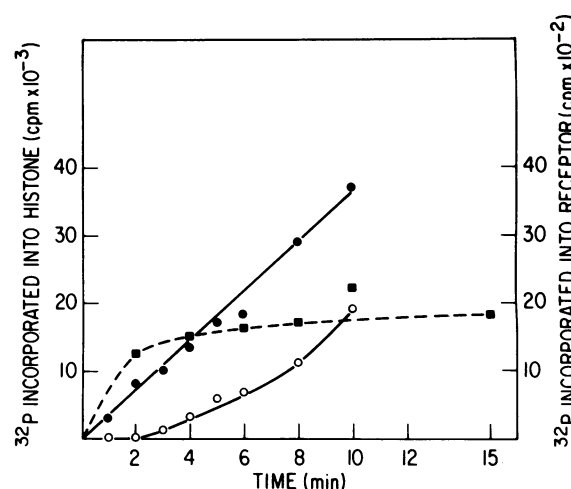


FIG. 1. Histone phosphorylation by the insulin-dependent receptor kinase with and without preincubation with ATP. Wheat germ agglutinin-agarose eluate (10  $\mu$ l) prepared from 3T3-L1 adipocyte membranes was incubated with insulin (1  $\mu$ g/ml) for 15 min at 23°C. [ $\gamma$ - $^{32}$ P]ATP (30 cpm/fmol) was then added for 6 min to one set of tubes (●) and an equal volume of  $H_2O$  was added to the other set (○). At 6 min, histone was added to the former series and histone and [ $\gamma$ - $^{32}$ P]ATP were added to the latter. At the indicated times thereafter, the reactions were terminated by the addition of trichloroacetic acid. The  $^{32}$ P incorporated in the absence of histone at each time point (220 cpm/min) was subtracted. In a separate experiment, 10  $\mu$ l of the wheat germ agglutinin-agarose eluate was incubated with or without insulin (2  $\mu$ g/ml) for 15 min at 23°C. [ $\gamma$ - $^{32}$ P]ATP (40 cpm/fmol) was then added (20  $\mu$ M) and the reaction was terminated, at the times indicated, by the addition of trichloroacetic acid. The  $^{32}$ P that was trichloroacetic-acid precipitable at time zero was subtracted from each set of reactions. The difference between the  $^{32}$ P incorporated in the presence and absence of insulin is presented (■). Parallel radioautograms of samples subjected to Na-DodSO<sub>4</sub>/polyacrylamide gel electrophoresis indicated that the only substrate for insulin-dependent tyrosine phosphorylation detected in the wheat germ agglutinin-agarose preparation was the 90,000-dalton insulin receptor subunit.

Activation by ATP and disappearance of the initial lag in exogenous substrate phosphorylation occurred within the time period required for insulin-dependent receptor phosphorylation (Fig. 1; Table 1). In the experiments presented in Table 1, the insulin-dependent phosphorylation of the insulin receptor in the wheat germ agglutinin-agarose eluate was complete by 6 min whereas phosphorylation of the affinity-purified material was not complete until 30 min had elapsed. Thus, the enzyme is maximally activated by preincubation with insulin and ATP. The activation seen after this preincubation (assessed in the presence or absence of insulin) will depend upon the extent to which previously unphosphorylated receptor becomes phosphorylated during the protein kinase assay (see ref. 3).

The insulin-stimulated protein kinase activity is stable for 30 min at 23°C at pH 5.0–6.0. This makes it possible to investigate the activity of the modified receptor after discharge of bound insulin by rapid gel filtration at pH 6.0. In the experiment shown in Fig. 4, aliquots of insulin-sensitive receptor kinase were incubated with either ATP, ATP plus insulin, insulin alone, or no addition. Each sample was then adjusted to pH 6.0, filtered, and assayed for phosphorylation of tyrosine residues on histone at pH 7.4. Enzyme that had been exposed to insulin plus ATP emerged from the column active and insulin-insensitive. On the other hand, untreated enzyme or enzyme exposed to either insulin or ATP alone was recovered in its original, insulin-dependent state. The insulin-independent activity exhibited by the phosphorylated receptor cannot be attributed simply to retention of bound insulin. When the receptor was incubated with

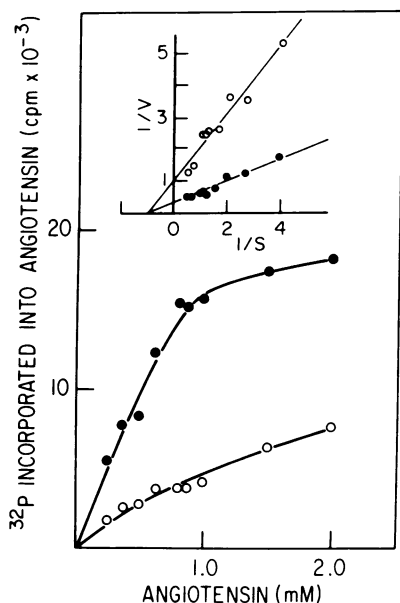


FIG. 2. Phosphorylation of angiotensin by the insulin receptor protein kinase with and without preincubation with ATP. Wheat germ agglutinin-agarose eluate derived from solubilized human placental membranes (5  $\mu$ l) was incubated for 20 min at 23°C with insulin at 1  $\mu$ g/ml. One set of tubes then received 20  $\mu$ M [ $\gamma$ - $^{32}$ P]ATP (21 cpm/fmol) for 6 min (●) and the other set received an equivalent volume of H<sub>2</sub>O (○). At 6 min, angiotensin at the indicated concentrations was added to the former series of tubes and both [ $\gamma$ - $^{32}$ P]ATP and angiotensin were added to the latter. Reactions were for 5 min. The  $^{32}$ P incorporated into angiotensin was assayed by the phosphocellulose paper procedure. (Inset) Reciprocal plot of the data.

ATP and  $^{125}$ I-labeled insulin (1  $\mu$ g/ml, 77 cpm/ng) for 15 min at 23°C and then adjusted to pH 6.0 and filtered, 0.3% of the input  $^{125}$ I-labeled insulin was detected in the fractions containing receptor. This amount of residual, bound  $^{125}$ I-labeled

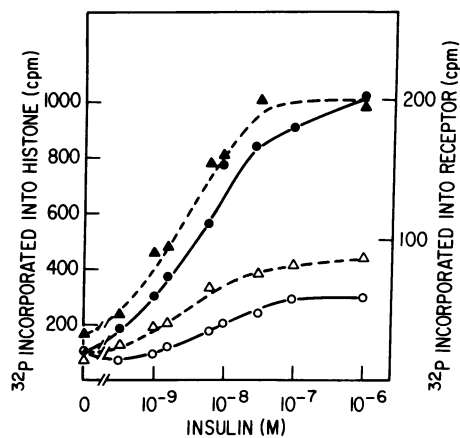


FIG. 3. Effect of insulin on activation by ATP and on phosphorylation of the receptor. Four sets of tubes containing wheat germ agglutinin-agarose eluate (2  $\mu$ l) prepared from human placenta were incubated with the indicated concentrations of insulin for 60 min at 23°C. [ $\gamma$ - $^{32}$ P]ATP (20  $\mu$ M; 18.2 cpm/fmol) was then added for 5 min to set 1 ( $\Delta$ ) or for 15 min to set 2 ( $\blacktriangle$ ). These incubations were then terminated by the addition of NaDodSO<sub>4</sub> sample buffer, and the  $^{32}$ P incorporated into the 90,000-dalton protein was assessed as described in the legend to Table 1. Set 3 (●) received [ $\gamma$ - $^{32}$ P]ATP for 10 min followed by histone for 5 min and set 4 (○) received [ $\gamma$ - $^{32}$ P]ATP and histone at the same time for 5 min. Reactions were terminated as above and the  $^{32}$ P incorporated into histone was assessed by NaDodSO<sub>4</sub>/polyacrylamide gel electrophoresis.

Table 1. Activation of insulin receptor kinase by preincubation with ATP

Preincubation with ATP, min	$^{32}$ P incorporation, fmol		
	Histone		Receptor
	With insulin	No insulin	
Exp. 1: wheat germ agglutinin-agarose eluate			
0	56.0	23.8	—
0.5	55.6	23.4	3.3
1	68.4	47.2	6.7
6	99.6	94.3	13.2
Exp. 2: affinity-purified receptor			
0	85.2	33.4	
30	278.8	405.9	

In Exp. 1, 50  $\mu$ l of the wheat germ agglutinin-agarose eluate prepared from solubilized 3T3-L1 adipocyte membranes was incubated with insulin (1  $\mu$ g/ml) for 15 min followed by the addition, for the indicated times, of 20  $\mu$ M [ $\gamma$ - $^{32}$ P]ATP (40 cpm/fmol) in the standard reaction mixture. The receptor was then resolved from both ATP and insulin by gel filtration at pH 6.0. Each fraction (60  $\mu$ l) from the column was divided into three 20- $\mu$ l aliquots. One portion was incubated with insulin (1  $\mu$ g/ml) for 15 min and then assayed for histone phosphorylation for 10 min in the standard reaction mixture containing 20  $\mu$ M [ $^{32}$ P]ATP (40 cpm/fmol). Another was assayed in the same fashion but without exposure to insulin, and the third was subjected directly to NaDodSO<sub>4</sub>/polyacrylamide gel electrophoresis. All reactions were terminated by the addition of NaDodSO<sub>4</sub> sample buffer. Following staining and destaining, gels were heated at 55°C for 1 hr in 1 M NaOH, washed, dried, and subjected to radioautography. The bands corresponding to histone or to the 90,000-dalton subunit of the receptor (in fractions that had not been assayed for histone kinase activity) were excised and assayed for radioactivity by liquid scintillation spectrometry. In Exp. 2, 35  $\mu$ l of affinity-purified placental insulin receptor was treated as in Exp. 1 except that the enzyme was preincubated for 30 min with unlabeled ATP (25  $\mu$ M) and 30  $\mu$ l of each fraction (total volume, 60  $\mu$ l) was assayed for histone kinase activity for 20 min. The  $^{32}$ P incorporated into tyrosine residues in histone was assessed by the trichloroacetic acid procedure. The  $^{32}$ P incorporated into protein in the absence of histone (<10% of that incorporated into histone) was subtracted from the data presented.

insulin (0.2 nM in the final protein kinase assay) did not alter the insulin sensitivity of the insulin-treated unphosphorylated receptor. When the same preparation of receptor was sedimented in a 5–20% sucrose gradient, the activated insulin-independent protein kinase activity had the same  $s_{20,w}$  as the untreated receptor (7). Although this peak of activity could bind added  $^{125}$ I-labeled insulin, none of the  $^{125}$ I-labeled insulin bound prior to centrifugation remained associated with the receptor. The final protein kinase assay using this material could not have contained more than 0.15 nM insulin. It remains possible, however, that a small amount of residual insulin associated with the receptor is bound differently to the phosphorylated receptor than it is to the unphosphorylated receptor and that this could be responsible for the apparent insulin “independence” of the phosphorylated receptor.

The ability of ATP to stimulate the receptor kinase activity with exogenous substrates also occurred with highly purified preparations of the placental insulin receptor (Table 1). The purified receptor was also rendered insulin-independent by the preincubation. In fact, kinase activity was frequently higher in the absence of added insulin. The observation that even in the adipocyte wheat germ agglutinin-agarose eluate, all of the tyrosine histone kinase activity cosedimented with the insulin receptor in 5–20% sucrose gradients in the presence of either 0.1 or 0.5 M NaCl makes it unlikely that ATP is activating a tyrosine kinase other than the insulin-receptor kinase in this relatively

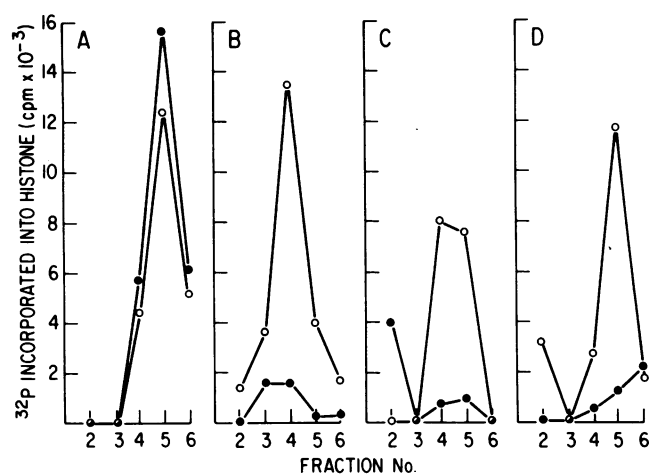


FIG. 4. Effect of preincubation with ATP and insulin on insulin receptor histone kinase activity after gel filtration at pH 6.0. Wheat germ agglutinin-agarose eluate (50  $\mu$ l) prepared from 3T3-L1 adipocyte membranes was incubated under the following conditions: (A) insulin (1  $\mu$ g/ml) for 15 min followed by 40  $\mu$ M ATP for 6 min; (B) insulin (1  $\mu$ g/ml) for 15 min; (C) no additions; (D) ATP (40  $\mu$ M) for 6 min. Each sample was then adjusted to pH 6.0 for 2 min and subjected to gel filtration at pH 6.0. Fractions (25  $\mu$ l) were assayed for histone kinase activity, with or without a 15-min exposure to insulin (1  $\mu$ g/ml). Incubations were for 10 min and the reactions were terminated by trichloroacetic acid precipitation. The [ $\gamma$ - $^{32}$ P]ATP used in the assay had a specific activity of 40 cpm/fmol. The recovery of total histone kinase activity (plus insulin) was approximately 40–50% under all conditions of preincubation.  $\circ$ , With insulin;  $\bullet$ , without insulin.

crude preparation. It is even less probable in the affinity-purified preparations of receptor.

Fractions containing the receptor that had been activated by exposure to [ $\gamma$ - $^{32}$ P]ATP and then filtered at pH 6.0 exhibited the expected single  $^{32}$ P-labeled 90,000-dalton band after Na-DodSO<sub>4</sub>/polyacrylamide gel electrophoresis and radioautography. The  $^{32}$ P-labeled receptor isolated by gel filtration did not catalyze the transfer of  $^{32}$ P from the 90,000-dalton subunit to histone when assayed in the presence or absence of Mg<sup>2+</sup> and Mn<sup>2+</sup> and unlabeled ATP. The possibility that ATP might be activating a contaminating trypsin-like protease which would act on the insulin-bound receptor and alter its properties was made unlikely by the observation that activation occurred to the same extent when the phosphorylation reaction was carried out in the presence of soybean trypsin inhibitor at 0.1 mg/ml. Sedimentation of the phosphorylated receptor kinase in the same position as the unmodified receptor kinase in sucrose gradients is consistent with this conclusion.

Finally, treatment of the insulin-independent phosphorylated receptor with 0.08 unit of bacterial alkaline phosphatase for 1 hr at 23°C in the presence of 0.1 M Tris-HCl, pH 8.0/0.1 M NaCl/0.1% Triton X-100/6% glycerol/78 mM MgCl<sub>2</sub> containing bovine serum albumin at 0.7 mg/ml resulted in 70% loss of  $^{32}$ P from the receptor. This dephosphorylation restored

the ability of insulin (1  $\mu$ g/ml) to stimulate histone phosphotransferase activity 1.7- to 1.8-fold when assayed after addition of 1.1 mM dithiothreitol, 60  $\mu$ M Na<sub>3</sub>VO<sub>4</sub>, 22 mM sodium phosphate (pH 7.4), and 4.0 mM *p*-nitrophenyl phosphate to inhibit phosphatase activity. Thus, dephosphorylation of the phosphorylated insulin receptor renders it dependent upon insulin for optimal activity.

## DISCUSSION

Although these results do not preclude the possibility that an ATP-dependent modification other than phosphorylation of tyrosine residues on the receptor is responsible for altering the properties of the receptor kinase, they are consistent with the proposition that insulin-dependent phosphorylation of the receptor stimulates the ability of this kinase to phosphorylate exogenous substrates. It may be that receptor phosphorylation, catalyzed by an intramolecular reaction involving an insulin-bound receptor, is responsible for insulin's ability to stimulate phosphotransferase reactions with exogenous substrates. Thus, the insulin-bound receptor would phosphorylate itself, altering its conformation so that the oligomer gains the capacity to catalyze the phosphorylation of exogenous substrates. The observation that the modified receptor remains active after removal of sufficient insulin to render the unphosphorylated receptor insulin-sensitive suggests that enzymatic dephosphorylation, not simply dissociation of bound insulin, may be involved in terminating the insulin signal.

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1. Kasuga, M., Karlsson, F. A. & Kahn, C. R. (1982) *Science* **215**, 185–187.
2. Kasuga, M., Zick, Y., Blithe, D. L., Karlsson, F. A., Haring, H. U. & Kahn, C. R. (1982) *J. Biol. Chem.* **257**, 9891–9894.
3. Petruzzelli, L. M., Ganguly, S., Smith, C. J., Cobb, M. H., Rubin, C. S. & Rosen, O. M. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6792–6796.
4. Kasuga, M., Fujiti-Yamaguchi, Y., Blithe, D. L. & Kahn, C. R. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2137–2141.
5. Avruch, J., Nemenoff, R. A., Blackshear, P. J., Pierce, M. W. & Osathanondh, R. (1982) *J. Biol. Chem.* **257**, 15162–15166.
6. Rubin, C. S., Hirsch, A., Fung, C. & Rosen, O. M. (1978) *J. Biol. Chem.* **253**, 7570–7578.
7. Siegel, T. W., Ganguly, S., Jacobs, S., Rosen, O. M. & Rubin, C. S. (1981) *J. Biol. Chem.* **256**, 9266–9273.
8. Bensadoun, A. & Weinstein, D. (1976) *Anal. Biochem.* **70**, 241–250.
9. Cooper, J. A. & Hunter, T. (1981) *Mol. Cell Biol.* **1**, 165–178.
10. Casnellie, J. E., Harrison, M. L., Pike, L. J., Hellstrom, K. E. & Krebs, E. G. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 282–286.
11. Glass, D. B., Masaracchia, R. A., Feramisco, J. R. & Kemp, B. E. (1978) *Anal. Biochem.* **87**, 566–575.
12. Wong, T. W. & Goldberg, A. R. (1983) *J. Biol. Chem.* **258**, 1022–1025.
13. Stadtmauer, L. & Rosen, O. M. (1983) *J. Biol. Chem.*, in press.