Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 7885–7889, September 1992
Biochemistry

Catalysis of serine and tyrosine autophosphorylation by the human insulin receptor

K. BALTENSPERGER*, R. E. LEWIS*†, C.-W. WOON*‡, P. VISSAVAJJHALA§¶, A. H. ROSS§, AND M. P. CZECH*‖

*Program in Molecular Medicine and Department of Biochemistry and Molecular Biology, University of Massachusetts Medical Center, Worcester, MA 01605; and §Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, MA 01545

Communicated by Edwin G. Krebs, May 4, 1992 (received for review February 10, 1992)

ABSTRACT **The protein kinase activity of human insulin receptors purified from Sf9 insect cells after infection with a recombinant baculovirus was evaluated. The following experimental observations led to the unexpected conclusion that this receptor protein catalyzes both serine and tyrosine autophosphorylation at significant stoichiometries. (*i*) Phosphorylation of lectin-purified insulin receptors with [γ-^{32}P]ATP resulted in rapid receptor tyrosine phosphorylation (7 mol of *P* per high-affinity binding site) and the delayed onset of insulin-stimulated receptor serine phosphorylation (about 7% of total phosphorylation). The tyrosine kinase inhibitor (hydroxy-2-naphthalenylmethyl)phosphonic acid (HNMPA), which has no effect on protein kinase C or cyclic AMP-dependent protein kinase activities, inhibited both the receptor serine and tyrosine phosphorylation. (*ii*) Phosphorylation of a synthetic peptide substrate composed of insulin receptor residues 1290–1319 on serines-1305/1306 by partially purified insulin receptors was also inhibited by HNMPA. (*iii*) Insulin receptors sequentially affinity-purified on immobilized wheat germ agglutinin and immobilized insulin showed no apparent contaminant proteins on silver-stained SDS/polyacrylamide gels yet catalyzed autophosphorylation on receptor serine and tyrosine residues when incubated with [γ-^{32}P]ATP. These results suggest that the catalytic site of the insulin receptor tyrosine kinase also recognizes receptor serine residues as substrates for the phosphotransfer reaction. Furthermore, insulin-stimulated receptor serine phosphorylation in intact cells may occur in part by an autophosphorylation mechanism subsequent to tyrosine phosphorylation of the insulin receptor.**

The insulin receptor is one of a number of growth factor receptors with intrinsic tyrosine kinase activity that can be activated upon binding of appropriate peptide ligands (1). Binding of insulin to its receptor also causes rapid phosphorylation of the tyrosine residues of the insulin receptor β subunit itself (2–4), which in turn further activates the tyrosine kinase activity (5, 6). In intact cells, insulin causes receptor phosphorylation on tyrosine as well as serine and threonine residues (2). Protein kinase C or protein kinases activated by this enzyme can phosphorylate the serine and threonine residues of the insulin receptor in intact cells (7, 8). Stimulation of serine phosphorylation of the insulin receptor by phorbol esters appears to correlate with inhibition of the insulin-stimulated tyrosine kinase activity of the receptor (8). Thus, phosphorylation of the serine and threonine residues of the insulin receptor may regulate insulin signaling by modulating receptor tyrosine kinase activity.

Several laboratory groups have reported the detection of an insulin-stimulated and receptor-associated serine/threonine kinase activity using the receptor itself as substrate, in partially purified (9–14) or affinity-purified (15) insulin receptor preparations. However, no physical separation of insulin receptor tyrosine kinase and insulin receptor-associated serine kinase activity has been reported. Furthermore, in our laboratory we have not succeeded in consistently separating the two activities (unpublished data). Therefore, we tested the hypothesis that insulin receptor serine phosphorylation is catalyzed by the receptor tyrosine kinase. The serine phosphorylation of partially purified insulin receptor and its serine kinase activity towards an exogenous substrate in the presence of the tyrosine kinase inhibitor (hydroxy-2-naphthalenylmethyl)phosphonic acid (HNMPA) were examined. The results show that the insulin receptor tyrosine kinase can catalyze its autophosphorylation on serine residues as well as the phosphorylation of a receptor-derived synthetic peptide containing the major serine phosphorylation site, Ser-1305/1306 (15). We conclude that insulin-activated serine phosphorylation of insulin receptors purified from cell extracts occurs by insulin receptor autophosphorylation.

MATERIALS AND METHODS

Materials. Sf9 cells, *Autographa californica* nuclear polyhedrosis virus (Ac*M*NPV) and transfer vector pVL1393 were provided by M. D. Summers (Texas Agricultural Experiment Station, Texas A & M University, College Station). Recombinant human insulin was provided by Ronald Chance (Lilly Research Laboratories). Monoiodinated [(125I)iodotyrosine-A14]insulin (2000 Ci/mmol; 1 Ci = 37 GBq) was from Amersham. Synthetic fragments of the insulin receptor were used: insulin receptor-(1155–1165) peptide (RDIYETDYYRK in single-letter code) and insulin receptor-(1290–1319) peptide (LEMEFEDMENVPLDRSSHCQREEAGGRDGG) (16).

Expression of Human Insulin Receptor in Sf9 Cells. The insulin receptor precursor cDNA (17) was cloned into the baculovirus transfer vector pVL1393 (18) by standard procedures (19). Recombinant virus was produced, isolated, and titrated as described (20, 21). The number of insulin receptor high-affinity binding sites was assayed by monoradioiodinated insulin binding to infected cells (22) or to soluble receptor (23). Scatchard analysis of the binding data was performed by using the program LIGAND (24).

Insulin Receptor Purification and Immunoprecipitation. Forty-eight hours after infection, Sf9 cells (10^9) were harvested by centrifugation (10 min at 1000 × *g*) and lysed at 4°C for 30 min in 100 ml of hypotonic buffer (10 mM Hepes, adjusted to pH 7.6 with NaOH/1 mM EDTA/10 μg of

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: HNMPA, (hydroxy-2-naphthalenylmethyl)phosphonic acid.

†Present address: Eppley Cancer Institute, University of Nebraska Medical Center, Omaha, NE 68198.
‡Present address: TSI Corporation, Innovation Drive, Worcester, MA 01605.
¶Present address: Vollum Institute, Oregon Health Science University, 3181 S.W. Sam Jackson Park Road, Portland, OR 97201.
‖To whom reprint requests should be addressed.

leupeptin per ml/1 mM phenylmethylsulfonyl fluoride). A crude membrane fraction was collected by centrifugation at 39,000 × *g* for 20 min and resuspended in 50 ml of the same buffer. Insulin receptor was solubilized by adding 3 volumes of buffer A (50 mM Hepes/NaOH, pH 7.6/5 mM EDTA/150 mM NaCl/1% (wt/vol) Triton X-100/1 mM phenylmethylsulfonyl fluoride/5 μg of leupeptin per ml/5 μg of aprotinin per ml) for 30 min. Insoluble material was removed by centrifugation as above, and insulin receptor was purified on immobilized wheat germ agglutinin (22), followed (where indicated) by insulin-conjugated agarose as described (15).

Insulin receptor expressed in Sf9 cells was immunoprecipitated from clarified cell lysates prepared in radioimmunoprecipitation assay (RIPA) buffer (0.5% Triton X-100/0.1% SDS/50 mM Hepes/NaOH, pH 7.6/1 M NaCl) with 0.5 μl of CT-1 ascites fluid per tube and 20 μl of Sepharose-coupled goat anti-mouse IgG (Cappel, Organon Teknika Corp.).

SDS/polyacrylamide gel electrophoresis (PAGE) (25) was performed under reducing conditions on 8% polyacrylamide gels. Silver staining was as described (26).

Autophosphorylation of Insulin Receptor and Peptide Phosphorylation. Insulin receptor (10 μl, 0.1 pmol) was incubated at 22°C for 15 min in a total volume of 45 μl containing (final concentrations) 30 mM Tris·HCl (pH 7.4), 0.1% Triton X-100, 100 mM NaCl, 10 mM $MgCl_2$, 3 mM $MnCl_2$, 100 μM sodium vanadate, 1 mM dithiothreitol, and 100 nM insulin, where indicated. Unless otherwise indicated, the reaction was started with 5 μl of 50 μM [γ-^{32}P]ATP (400 Ci/mmol) and was stopped after 30 min by addition of 50 μl of SDS/PAGE sample buffer and denaturation for 5 min at 100°C. One- and two-dimensional phosphoamino acid analysis of insulin receptor β subunit was performed on cellulose TLC plates after partial hydrolysis at 110°C in 6 M HCl for 90 min of material eluted from trypsinized gel slices as described (15, 27). Radioactivity in phosphotyrosine and phosphoserine spots was directly quantitated by exposing the thin-layer chromatograms on a Betascope (Betagen, Waltham, MA). The stoichiometry of overall insulin receptor phosphorylation was estimated from Cerenkov radiation contained in the insulin receptor β subunit after SDS/PAGE.

Phosphorylation and detection of synthetic insulin receptor-(1290–1319) peptide was modified from the description of Lewis *et al.* (15). Partially purified insulin receptor (200 μl; 2 pmol) was mixed in a total volume of 1.4 ml of 300 mM 3-(*N*-morpholino)propanesulfonic acid, pH 7.6/0.1% Triton X-100/100 mM NaCl/10 mM $MgCl_2$/3 mM $MnCl_2$/100 μM sodium vanadate/1 mM dithiothreitol/100 nM insulin where indicated. Two aliquots of 600 μl were phosphorylated in the presence or absence of 100 nM insulin by adding ATP to a final concentration of 50 μM for 1 hr at 22°C. Aliquots of 140 μl were then transferred to tubes containing the peptide, peptide and HNMPA, or neither (control) in a total volume of 40 μl. Reactions were started with 20 μl of 500 μM [γ-^{32}P]ATP (20 Ci/mmol) and stopped after 30 min with 84 μl of glacial acetic acid and incubation on ice for 10 min. Reaction mixtures were then applied to 3.5-ml columns of Dowex 1 1X4-100 anion-exchange resin equilibrated in 30% acetic acid. Radioactive peptide was eluted by adding 1-ml aliquots of 30% acetic acid. The fractions were analyzed for radioactivity, pooled (3 ml), and applied on 0.85-ml SepPak C_{18} cartridges (Waters, Millipore) primed with 5 ml of acetonitrile and 10 ml of 0.1% CF_3COOH. Each column was then washed with 10 ml of 0.1% CF_3COOH and eluted with 1.5 ml of 50% acetonitrile/0.1% CF_3COOH in water. The eluate was lyophilized and then separated in two dimensions by thin-layer electrophoresis and chromatography (15). For phosphoamino acid analysis, the phosphopeptide was eluted from the thin-layer plate with 30% formic acid.

RESULTS

Human insulin receptor was expressed in Sf9 cells by using a recombinant baculovirus. Extracts from infected cells were partially purified on immobilized wheat germ agglutinin, incubated with [γ-^{32}P]ATP for 30 min and then analyzed on SDS/PAGE. The autoradiogram depicted in Fig. 1 shows two major bands of apparent M_r 180,000 and 86,000. Insulin (100 nM) selectively stimulated the phosphorylation of the apparent M_r 86,000 species 1.7 ± 0.3-fold (n = 7). Maximum stoichiometry of phosphorylation as assayed by incubation with 500 μM [γ-^{32}P]ATP for 30 min at 22°C was 7 mol of phosphorus per insulin receptor high-affinity binding site (K_d = 0.5 nM). The monoclonal antibody CT-1 (28) immunoprecipitated both phosphoproteins (not shown), suggesting that the apparent M_r = 180,000 and 86,000 bands represent the uncleaved proreceptor form and the β subunit of the proteolytically processed insulin receptor, respectively. When cell extracts were sequentially purified on immobilized wheat germ agglutinin and immobilized insulin and then incubated for 30 min in the presence of [γ-^{32}P]ATP, only a protein with apparent M_r 86,000 was labeled (Fig. 1). This protein was identified as the insulin receptor β subunit by its ability to undergo insulin-stimulated phosphorylation (2.5 ± 0.5-fold, n = 4) and by immunoprecipitation with CT-1 (not shown). Labeled proreceptor was absent in this preparation. Furthermore, using dissuccinimidyl suberate (29), monoradioiodinated insulin could be crosslinked to the α subunit of the proteolytically processed receptor but not to the proreceptor (not shown). These observations are consistent with the notion that the proreceptor lacks insulin sensitivity because of its inability to bind insulin (30) and therefore is not enriched by insulin affinity chromatography.

Phosphorylation of partially purified receptor occurred not only on tyrosine but also on serine residues in an insulin-stimulated manner (Fig. 2*A*). Interestingly, insulin-stimulated serine phosphorylation was also detectable in highly purified receptor preparations, prepared by insulin-agarose affinity chromatography (Fig. 2*A*). However, in contrast to the partially purified receptor preparation, insu-

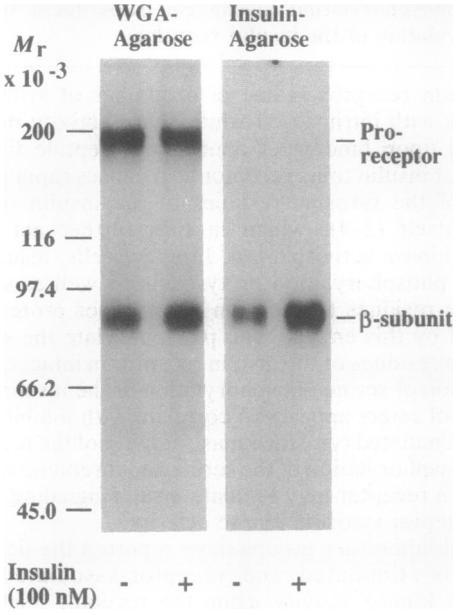

FIG. 1. Autophosphorylation of insulin receptor expressed in insect cells. Insulin receptor was purified from infected Sf9 cells on immobilized wheat germ agglutinin (WGA-Agarose), followed by insulin-agarose affinity chromatography (Insulin-Agarose). Eluates were phosphorylated in the absence or presence of 100 nM insulin and analyzed by SDS/PAGE and autoradiography, which is shown.

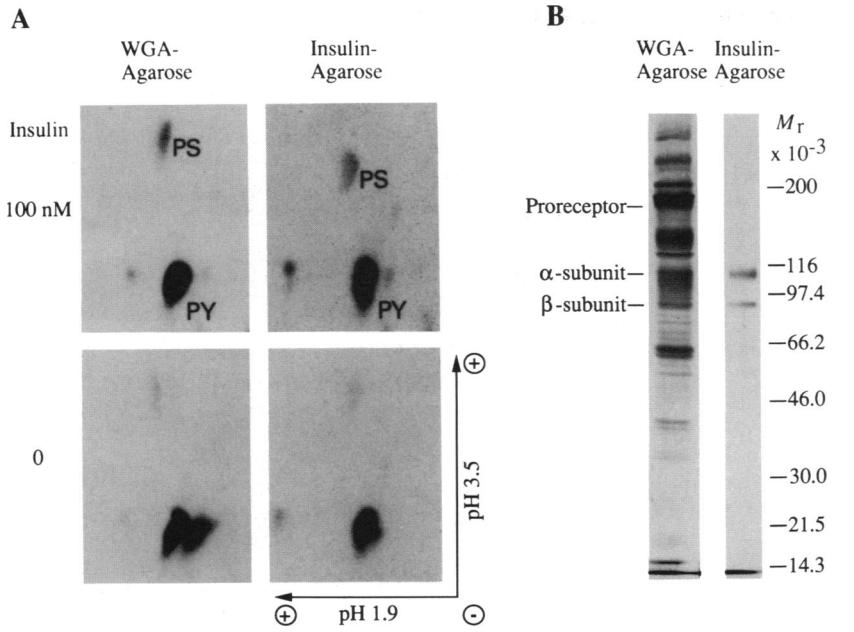

FIG. 2. Detection of phosphoserine in partially purified and highly purified insulin receptor. (*A*) Equal amounts (0.1 pmol) of partially purified (WGA-Agarose) or highly purified (Insulin-Agarose) insulin receptor were analyzed for their phosphoamino acid content or protein content. (*A*) Insulin receptor was autophosphorylated and processed for two-dimensional phosphoamino acid analysis of the β subunit. Phosphoamino acids were detected by autoradiography. (*B*) The insulin receptor preparations were analyzed for their protein content by SDS/PAGE (5–15% acrylamide) and silver staining. The stained bands for proreceptor and insulin receptor subunits are indicated based on the relative mobility of insulin receptor species extracted from [^{32}S]methionine-labeled infected cells (not shown) and ^{32}P-phosphorylated partially purified insulin receptor (see Fig. 1). The relative mobilities of unlabeled phosphoamino acid standards are indicated: PS, phosphoserine; PY, phosphotyrosine.

lin-agarose-purified receptor did not show any apparent contaminants when analyzed by silver staining (Fig. 2*B*). With respect to overall phosphorylation, the phosphoserine content of the β subunit was very similar in the two preparations (5.5% and 3.6% phosphoserine in the absence of insulin and 6.9% and 6.7% phosphoserine in the presence of insulin for partially purified and insulin-agarose-purified receptor, respectively). Furthermore, when CT-1 immunoprecipitates from clarified cell lysates were washed three times under stringent conditions (RIPA buffer, see *Materials and Methods*) and then incubated with [γ-^{32}P]ATP, 6.7% of total insulin receptor phosphorylation was retrieved as phosphoserine (not illustrated)—i.e., serine phosphorylation appears to occur with a stoichiometry of ≈0.5 mol *P* per high-affinity insulin binding site. This estimate is not exact because of the disproportionate rate of release and decomposition of phosphoserine and phosphotyrosine during acid hydrolysis of phosphopeptides (27).

Tyrosine phosphorylation of the insulin receptor precedes serine phosphorylation under the conditions of these experiments (Fig. 3). Serine phosphorylation of the β subunit is detectable only at about 8 min after the addition of [γ-^{32}P]ATP and is stimulated by insulin (Fig. 3, 32-min time point). These data are consistent with previous observations (31, 32) and suggest that tyrosine phosphorylation of the insulin receptor may be required for serine phosphorylation of the receptor to occur.

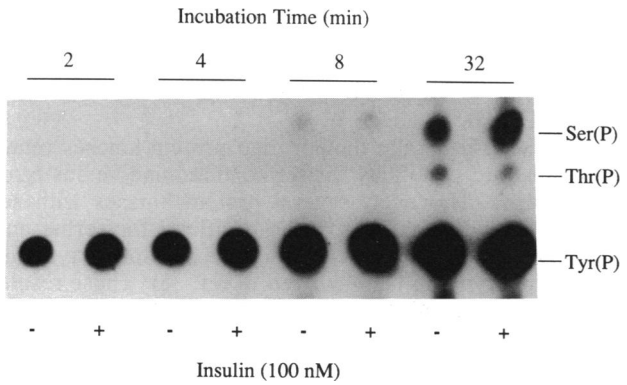

FIG. 3. Time course of tyrosine vs. serine phosphorylation of insulin receptor β subunit. Partially purified insulin receptor was phosphorylated in the absence (−) or presence (+) of 100 nM insulin for the times indicated. The phosphoamino acid composition of the β subunit was then analyzed by one-dimensional thin-layer electrophoresis. The autoradiograph of the thin-layer plate is shown. Ser(P), phosphoserine; Thr(P), phosphothreonine; Tyr(P), phosphotyrosine.

Taken together, the data in Figs. 1–3 suggested that hypothesis that insulin-stimulated insulin receptor serine phosphorylation is due to an intrinsic activity of the tyrosine kinase itself. We tested this possibility by using agents shown to be specific for inhibiting tyrosine kinases but not serine kinases. Among the four inhibitors tested [genistein (33), tyrphostin (34), athiazolidinedione (CGP 520 III) (35), and HNMPA (36)], only the latter inhibited insulin receptor tyrosine kinase activity specifically when compared with inhibition of kemptide phosphorylation by the catalytic subunit of cAMP-dependent protein kinase. Consistent with previous data (36), 300 μM HNMPA inhibited tyrosine kinase activity of autophosphorylated insulin receptor towards poly(Glu_4, Tyr) or insulin receptor-(1155–1165) peptide {[Tyr]1143–1153 in Shoelson *et al.* (37)} by 82% and 81%, respectively, whereas no effect on protein kinase A catalytic subunit was detectable (not shown). Autophosphorylation of insulin receptors in the presence of insulin was inhibited by 13% ± 4.6% (n = 6) when 300 μM HNMPA was added prior to the addition of [γ-^{32}P]ATP. As previously found (36), insulin receptor autophosphorylation appears to be less sensitive to the inhibitor than substrate phosphorylation. However, inhibition of insulin receptor autophosphorylation by 2.5 mM HNMPA was 48%, and still no inhibition of protein kinase A catalytic subunit activity was observed [98.6% ± 2.9% (n = 3) of control activity]. Importantly, HNMPA not only inhibited insulin receptor tyrosine phosphorylation but also effectively decreased the extent of insulin receptor serine phosphorylation at concentrations of 300 μM (Fig. 4*A*) and 2.5 mM (Fig. 4*B*). Moreover, the effect of HNMPA on serine phosphorylation was more pronounced than that on tyrosine phosphorylation and was similar to that seen for the inhibition of tyrosine kinase activity towards exogenous substrates.

Although the results in Fig. 4 are compelling, they do not conclusively rule out the possibility that the tyrosine kinase inhibitor decreased insulin receptor serine phosphorylation by inhibiting the phosphorylation of one or more tyrosine residues critical for exposing serine residues as substrates for a contaminating serine kinase. To test whether HNMPA inhibits serine phosphorylation directly by inhibiting the insulin receptor tyrosine kinase, the effect of HNMPA on the

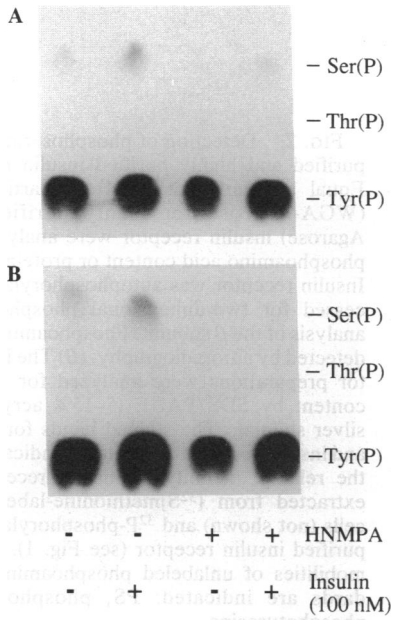

FIG. 4. Effect of HNMPA on insulin receptor tyrosine and serine phosphorylation. Insulin receptor was incubated with 50 μM [γ-^{32}P]ATP in the absence (−) or presence (+) of insulin. Where indicated, 300 μM (*A*) or 2.5 mM (*B*) HNMPA was included. The phosphoamino acid composition of the insulin receptor β subunit was analyzed as described in text. The autoradiograph is shown. Ser(P), phosphoserine; Thr(P), phosphothreonine; Tyr(P), phosphotyrosine.

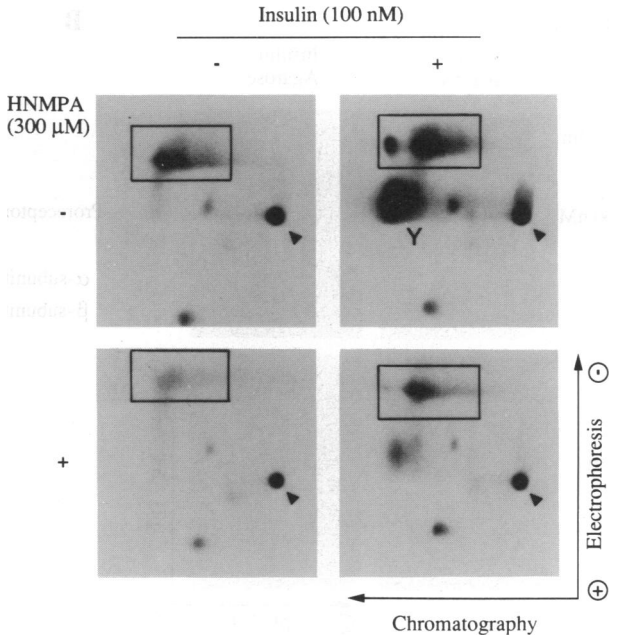

FIG. 5. Inhibition by HNMPA of insulin receptor-(1290–1319) peptide phosphorylation. Insulin receptor (0.2 pmol) was preincubated in the absence (Left) or presence (Right) of 100 nM insulin for 1 hr in the presence of 50 μM ATP and then was incubated with 1 mM insulin receptor-(1290–1319) peptide in the presence of [γ-^{32}P]ATP (100 μM) and in the presence (*Lower*) or absence (*Upper*) of 300 μM HNMPA. The phosphorylated peptide was isolated and analyzed in two dimensions by electrophoresis and chromatography as described in text. The developed thin-layer plates were exposed to x-ray film for 20 hr. The autoradiography of one of four independent experiments with similar results is shown. Quantitation of the insulin receptor-(1290–1319) phosphopeptide (boxed areas) was achieved by exposing the TLC plate to a Betascope. Y, species containing phosphotyrosine and tentatively identified as phosphotyrosine-containing insulin; arrowhead, origin of electrophoresis.

phosphorylation of insulin receptor-(1290–1319) peptide, composed of a known insulin receptor serine phosphorylation site (15) was examined. Phosphorylation of insulin receptor-(1290–1319) peptide by insulin receptors is stimulated in the presence of insulin (Fig. 5). When 300 μM HNMPA was added together with the peptide, phosphorylation of the peptide was reduced by 42% in the absence and by 47% in the presence of insulin, indicating that the serine kinase activity inhibited by HNMPA is indeed insulin-stimulated. In two experiments, the phosphopeptide was further analyzed for its phosphoamino acid content. Only phosphoserine could be detected (not shown), which is consistent with the sequence of insulin receptor-(1290–1319) peptide. The present data strongly favor the conclusion that the receptor tyrosine kinase itself catalyzes phosphorylation of both receptor serine residues (Figs. 2 and 4) and serine substrate peptide (Fig. 5).

DISCUSSION

The observation made in several laboratories (9–15) that partially or highly purified insulin receptors can catalyze insulin-activated phosphorylation of receptor serine as well as tyrosine residues is consistent with three possible hypotheses: (*i*) a novel serine kinase tightly bound to the receptor is activated upon insulin stimulation of the receptor tyrosine kinase, (*ii*) receptor tyrosine autophosphorylation allows a novel serine kinase tightly bound to the receptor to phosphorylate the receptor more rapidly, or (*iii*) the receptor tyrosine kinase itself can also phosphorylate directly certain receptor serine residues. Recent work in our laboratory (15) demonstrated that the receptor-associated serine kinase activity could also catalyze exogenous peptide substrate phosphorylation in an insulin-sensitive manner, thus eliminating the second hypothesis above. The present studies further demonstrate that the receptor-associated serine kinase activity is attenuated by the tyrosine kinase inhibitor HNMPA (Figs. 4 and 5). Although we cannot unequivocally rule out the possibility of an unusual serine kinase that is sensitive to HNMPA, the selectivity of HNMPA as a specific tyrosine kinase inhibitor is impressive. The activity of protein kinase A was unaffected by the inhibitor at concentrations 8-fold higher than those that inhibited the receptor serine kinase activity (Fig. 5). Consistently, HNMPA is also without effect on protein kinase C (36). Also, unlike other less selective tyrosine kinase inhibitors such as genistein or quercitin that exhibit some activity against serine kinases, HNMPA is not competitive with respect to ATP (36). Taken together, our data (Figs. 4 and 5) appear to eliminate the first hypothesis above and strongly favor the conclusion that the receptor tyrosine kinase itself catalyzes phosphorylation of both receptor serine residues (Figs. 2 and 4) and serine substrate peptide (Fig. 5).

It had been generally thought that protein kinases retain strict substrate specificity for serine/threonine versus tyrosine residues. However, several protein kinases with sequences similar to kinases with specificity for serine and threonine have recently been found to also catalyze tyrosine autophosphorylation (summarized in ref. 38). These results are strikingly exemplified by the mammalian Erk-1 and Erk-2 gene products that autophosphorylate on both tyrosine and threonine residues even when expressed in bacteria (39, 40). Our studies extend the conceptual implications of those observations in that they indicate analogous properties for a tyrosine kinase. Thus, the insulin receptor tyrosine kinase appears to catalyze serine and tyrosine autophosphorylation.

Serine phosphorylation of exogenous substrates by insulin receptor appears to be several orders of magnitude slower than phosphorylation of tyrosines on such substrates as

poly(Glu^{80}Tyr^{20}) or insulin receptor-1155–1165) peptide, which is derived from the major insulin receptor tyrosine phosphorylation site (not illustrated). These observations are consistent with the data of Walker *et al.* (41), who examined the hydroxylamino acid selectivity of the insulin receptor tyrosine kinase using the insulin receptor synthetic peptide substrate Arg-Arg-Leu-Ile-Glu-Asp-Ala-Glu-*Tyr*-Ala-Ala-Arg-Gly and serine/threonine analogues thereof. In a conventional kinase assay, no phosphorylation of the analogues was detectable. Similarly, phosphorylation of insulin receptor-(1290–1319) peptide was not detectable in the present studies in the same type of assay (not shown). Only when the substrate phosphopeptide was isolated and separated in two-dimensions could the small amount of labeled phosphopeptide be identified and quantitated (Fig. 5).

The extent of receptor serine autophosphorylation observed in these studies is less than the level of serine phosphorylation observed for insulin receptors isolated from ^{32}P-labeled, insulin-stimulated cells (2–4), including Sf9 cells infected with recombinant baculovirus (42). Thus, it is likely that in intact cells, other insulin-stimulated serine kinase activities are involved in receptor phosphorylation. However, the characteristics of the two processes are similar. Both are delayed relative to the insulin-sensitive receptor tyrosine phosphorylation and both occur at sites in the carboxyl-terminal region of the insulin receptor (7, 15). Although the role of receptor serine and threonine phosphorylation is not yet unequivocally defined, these similarities suggest that, in addition to receptor phosphorylation by cellular kinases, insulin-activated receptor autophosphorylation may be important in the regulation of insulin receptor function.

We are indebted to Dr. J. Whittaker (Univ. of Chicago) for providing the human insulin receptor precursor cDNA. We also thank Dr. K. Siddle (Univ. of Cambridge, U.K.) for providing the monoclonal antibody CT-1 and Dr. W. J. Greenlee (Merck Sharp & Dohme) for providing HNMPA. This work was supported by Research Grant DK30648. K. B. was supported by postdoctoral fellowships from the Swiss National Science Foundation and the Juvenile Diabetes Foundation International.

1. Ullrich, A. & Schlessinger, J. (1990) *Cell* **61**, 203–212.
2. Kasuga, M., Zick, Y., Blith, D. L., Karlsson, F. A., Häring, H. U. & Kahn, C. R. (1982) *J. Biol. Chem.* **257**, 9891–9894.
3. White, M. F., Takayama, S. & Kahn, C. R. (1985) *J. Biol. Chem.* **260**, 9470–9478.
4. Pang, D. T., Sharma, B. R., Shafer, J. A., White, M. F. & Kahn, C. R. (1985) *J. Biol. Chem.* **260**, 7231–7136.
5. Rosen, O. M., Herrera, R., Olowe, Y. H., Petruzzelli, L. M. & Cobb, M. H. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3237–3240.
6. Yu, K.-T. & Czech, M. P. (1984) *J. Biol. Chem.* **259**, 5277–5286.
7. Lewis, R. E., Cao, L., Perregaux, D. & Czech, M. P. (1990) *Biochemistry* **29**, 1807–1813.
8. Takayama, S., White, M. F. & Kahn, C. R. (1988) *J. Biol. Chem.* **263**, 3440–3447.
9. Zick, Y., Grunberger, G., Podskalny, J. M., Moncada, J. M., Taylor, S. I., Gorden, P. & Roth, J. (1983) *Biochem. Biophys. Res. Commun.* **116**, 1129–1135.
10. Ballotti, R., Kowalski, A., Le Marchand-Brustel, Y. & Van Obberghen, E. (1986) *Biochem. Biophys. Res. Commun.* **139**, 179–185.
11. Smith, D. M., King, M. J. & Sale, G. J. (1988) *Biochem. J.* **250**, 509–519.
12. Biener, Y. & Zick, Y. (1990) *Eur. J. Biochem.* **194**, 243–250.
13. Tavaré, J. M. & Dickens, M. (1991) *Biochem. J.* **274**, 173–179.
14. Pillay, T. S. & Siddle, K. (1991) *Biochem. Biophys. Res. Commun.* **179**, 962–971.
15. Lewis, R. E., Wu, G. P., MacDonald, R. G. & Czech, M. P. (1990) *J. Biol. Chem.* **265**, 947–954.
16. Ebina, Y., Ellis, L., Jarnagin, K., Edery, M., Graf, L., Clauser, E., Ou, J.-h., Masiarz, F., Kan, Y. W., Goldfine, I. D., Roth, R. A. & Rutter, W. J. (1985) *Cell* **40**, 747–758.
17. Whittaker, J., Okamoto, A. K., Thys, R., Bell, G. I., Steiner, D. F. & Hofmann, C. A. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5237–5241.
18. Luckow, V. A. & Summers, M. D. (1989) *Virology* **170**, 31–39.
19. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
20. Summers, M. D. & Smith, G. E. (1987) *A Manual of Methods of Baculovirus Vectors and Insect Cell Culture Procedures* (Texas Agric. Exp. Sta. Bull. No. 1555).
21. Fung, M.-C., Chiu, K. Y. M., Weber, T., Chang, T.-W. & Chang, N. T. (1988) *J. Virol. Methods* **19**, 33–42.
22. Paul, J., Tavaré, J., Denton, R. M. & Steiner, D. F. (1990) *J. Biol. Chem.* **265**, 13074–13083.
23. Harrison, L. C. & Itin, A. (1980) *J. Biol. Chem.* **255**, 12066–12072.
24. Munson, P. J. & Rodbard, D. (1980) *Anal. Biochem.* **107**, 220–239.
25. Laemmli, U. K. (1979) *Nature (London)* **227**, 670–685.
26. Merryl, C. R., Dunan, M. L. & Goldman, D. (1981) *Anal. Biochem.* **110**, 201–207.
27. Cooper, J. A., Sefton, B. M. & Hunter, T. (1983) *Methods Enzymol.* **99**, 387–402.
28. Prigent, S. A., Stanley, K. K. & Siddle, K. (1990) *J. Biol. Chem.* **265**, 9970–9977.
29. Pilch, P. F. & Czech, M. P. (1980) *J. Biol. Chem.* **255**, 1722–1731.
30. Yoshimasa, Y., Seino, S., Whittaker, J., Kakehi, T., Kosaki, A., Kuzuya, H., Imura, H., Bell, G. I. & Steiner, D. F. (1988) *Science* **240**, 784–787.
31. Smith, D. M. & Sale, G. J. (1988) *Biochem J.* **256**, 903–909.
32. Tavaré, J. M., Clack, B. & Ellis, L. (1991) *J. Biol. Chem.* **266**, 1390–1395.
33. Akiyama, T., Ishida, J., Nakagawa, S., Ogawara, H., Watanabe, S.-i., Itoh, N., Shibuya, M. & Fukami, Y. (1987) *J. Biol. Chem.* **262**, 5592–5595.
34. Gazit, A., Yaish, P., Gilon, C. & Levitzki, A. (1989) *J. Med. Chem.* **32**, 2344–2352.
35. Geissler, J. F., Traxler, P., Regenass, U., Murray, B. J., Roesel, J. L., Meyer, T., McGlynn, E., Storni, A. & Lydon, N. B. (1990) *J. Biol. Chem.* **265**, 22255–22261.
36. Saperstein, R., Vicario, P. P., Stout, H. V., Brady, E., Slater, E. E., Greenlee, W. J., Ondeyka, D. L., Patchett, A. A. & Hangauer, D. (1989) *Biochemistry* **28**, 5694–5701.
37. Shoelson, S. E., White, M. F. & Kahn, C. R. (1989) *J. Biol. Chem.* **264**, 7831–7836.
38. Lindberg, R. A., Quinn, A. M. & Hunter, T. (1992) *Trends Biochem. Sci.* **17**, 114–119.
39. Crews, C. M., Alessandrini, A. A. & Erikson, R. L. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 8845–8849.
40. Seger, R., Ahn, N. G., Boulton, T. G., Yancopoulos, G. D., Panayotatos, N., Radziejewska, E., Ericsson, L., Bratlien, R. L., Cobb, M. H. & Krebs, E. G. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 6142–6146.
41. Walker, D. H., Kuppuswamy, D., Visvanathan, A. & Pike, L. (1987) *Biochemistry* **26**, 1428–1433.
42. Baltensperger, K., Woon, C.-W., Vissavajjhala, P., Ross, A. H. & Czech, M. P. (1991) *J. Cell. Biochem.* **15B**, 54 (abstr.).