Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 3972–3976, June 1987
Biochemistry

Insulin stimulates a membrane-bound serine kinase that may be phosphorylated on tyrosine

(adipocyte)

KIN-TAK YU, NAZER KHALAF, AND MICHAEL P. CZECH

Department of Biochemistry, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01605

Communicated by Robert L. Hill, February 5, 1987

ABSTRACT **Triton X-100-solubilized high-density microsomes from insulin-treated rat adipocytes exhibit a marked increase in serine/threonine and tyrosine kinase activities toward exogenous histone when compared to controls. The insulin-dependent activation of microsomal histone kinase activities occurs within the physiological range of hormone concentrations (ED_{50} = 0.6 nM). The hormone-enhanced histone phosphorylation by the high-density microsomes appears to be catalyzed by two distinct kinases, based on their differential interaction with wheat germ agglutinin-agarose. The insulin-sensitive serine/threonine kinase is not retained by the lectin column, whereas the tyrosine kinase appears to be a glycoprotein as evidenced by its adsorption to the immobilized lectin. The insulin-stimulated serine/threonine kinase exhibits preferential phosphorylation of histone and Kemptide (synthetic Leu-Arg-Arg-Ala-Ser-Leu-Gly) compared to a number of other peptide substrates. The substrate specificity of this serine/threonine kinase shows that it is distinct from the kinases that phosphorylate ribosomal protein S6, casein, phosvitin, ATP citrate lyase, and glycogen synthase and from multifunctional calmodulin-dependent, cAMP- and cGMP-dependent, and Ca^{2+}/phospholipid-dependent protein kinases. Furthermore, 22% of the insulin-sensitive serine/threonine kinase activity can be adsorbed by monoclonal anti-phosphotyrosine antibodies immobilized on agarose. Its adsorption is specifically inhibited by excess free phosphotyrosine but not phosphoserine or phosphothreonine. The data suggest that this insulin-stimulated serine/threonine kinase in adipocyte high-density microsomes is tyrosine-phosphorylated, consistent with the hypothesis that the stimulatory action of insulin on this kinase may be mediated by tyrosine phosphorylation.**

The regulatory action of insulin on cellular metabolism involves the modulation of the serine or threonine phosphorylation states of key regulated enzymes (1). Insulin action leads to increased phosphorylation of several cellular proteins and to decreased phosphorylation of others (2). It is thought that, in order to mediate such actions, insulin regulates cellular serine/threonine kinase or phosphatase activities or both. This hypothesis is exemplified by recent reports that the serine phosphorylation of ribosomal protein S6 is markedly increased by insulin in 3T3-L1 fibroblasts (3–5). Addition of insulin to these cells results in increased S6 kinase activity in cell extracts. It has also been shown that S6 phosphorylation in oocytes is markedly enhanced in an insulin-dependent fashion after microinjection of purified insulin receptor with or without prebound hormone (6). However, the molecular mechanism(s) by which insulin modifies such protein kinase and phosphatase activities is not understood (7).

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.

The insulin receptor is a tyrosine kinase that is activated severalfold upon binding of insulin (8–11). Although the cellular substrate or substrates for the insulin receptor kinase have yet to be identified, it has been suggested that cellular signaling by insulin may be transmitted by the intrinsic tyrosine kinase activity of the receptor (12, 13). It is possible that the insulin receptor kinase may mediate biological signals through modulation of one or more serine kinases or phosphatases directly or indirectly by tyrosine phosphorylation (5, 14).

In this report we present direct evidence that a serine kinase distinct from the ribosomal protein S6 kinase is markedly stimulated in a specific cellular membrane fraction derived from insulin-treated rat adipocytes. Further, the hypothesis that this membrane-associated serine kinase may be activated by insulin-promoted tyrosine phosphorylation is supported by the immunoreactivity of this serine kinase activity with monoclonal antibodies directed against phosphotyrosine.

MATERIALS AND METHODS

Cell Isolation and Incubation. Male rats of 100–125 g were used (Sprague–Dawley strain, Taconic Farms, Germantown, NY). Adipocytes were prepared from the epididymal fat pads as described by Rodbell (15) in Krebs–Ringer/Hepes buffer (pH 7.4) containing bovine serum albumin (30 mg/ml) and sodium pyruvate (2 mM). Fat cells were equilibrated in the same buffer without collagenase at 37°C for 10 min. After equilibration, adipocytes were incubated in the absence or presence of insulin (0.1–100 nM) at 37°C for 10 min and then homogenized according to the procedure described below.

Preparation of High-Density Microsomes (HDM). Fat cells were homogenized in 10 mM Tris·HCl, pH 7.4/225 mM sucrose/5 mM EDTA/10 mM NaF/200 μM sodium vanadate/10 mM sodium pyrophosphate/1 mM phenylmethylsulfonyl fluoride. HDM were prepared by differential centrifugation as described by McKeel and Jarett (16) with some modifications (17). Membranes were suspended in buffer A (25 mM Hepes, pH 7.4/100 mM NaF/200 μM sodium vanadate/2 mM dithiothreitol/1 mM phenylmethylsulfonyl fluoride). Membrane protein concentrations were determined by Coomassie blue G-250 binding (Bio-Rad protein assay) and adjusted to 0.5–1 mg/ml.

Assay of Protein Kinase Activities in Adipocyte HDM. The suspended HDM were solubilized in 1% (vol/vol) Triton X-100 at 4°C for 30 min. Twenty microliters of the solubilized membrane preparation was mixed with 5 μl of histone type V-S (Sigma). Phosphorylation was initiated by the addition of 8 μl of labeling mixture containing 17 mM $MgCl_2$, 5 mM $MnCl_2$, 17 mM dithiothreitol, and 0.33 mM [γ-^{32}P]ATP (specific activity 100 μCi/nmol; 1 Ci = 37 GBq). The reaction was terminated after 10 min at 22°C by the addition of 0.1 ml

Abbreviation: HDM, high-density microsome(s).

of electrophoresis sample buffer containing 100 mM dithiothreitol and 10% $NaDodSO_4$. The samples were boiled for 5 min and then electrophoresed in a $NaDodSO_4$/10% polyacrylamide gel. After electrophoresis, the proteins were fixed and the gel was autoradiographed. The amount of ^{32}P radioactivity incorporated into histone was quantitated either by Cerenkov counting of the excised protein band or by densitometric scanning of the autoradiogram.

Immunoadsorption to Monoclonal Anti-Phosphotyrosine Antibodies. Triton X-100-solubilized HDM from control and insulin-treated adipocytes were mixed with 10 μl of Affi-Gel 10 derivatized with monoclonal anti-phosphotyrosine immunoglobulin (14 mg/ml, kindly provided by J. Klarlund of this laboratory) at 4°C for 2 hr. The utility of this anti-phosphotyrosine antibody preparation for immunoprecipitation of phosphotyrosine-containing proteins has been reported (18). The resin was then sedimented at 600 × *g* and washed three times with 1 ml of buffer A containing 1% Triton X-100. The adsorbed materials were eluted in 40 μl of buffer A containing 1% Triton X-100 and 10 mM *p*-nitrophenyl phosphate. Twenty microliters of the eluate and the remaining resin were used for phosphorylation experiments.

Phospho Amino Acid Analysis. Extraction of ^{32}P-labeled proteins from the $NaDodSO_4$/polyacrylamide gel and analysis of their phospho amino acid contents were done as described (19).

RESULTS

Insulin-Stimulated Histone Kinase Activity in Adipocyte HDM. The phosphorylation of exogenous histone by HDM prepared from control and insulin-treated rat adipocytes is depicted in Fig. 1. As evidenced in the autoradiogram, the ^{32}P-labeling of histone type V-S is increased ≈2-fold in the insulin-treated group when compared to the control (Fig. 1*A*). Direct quantitation by Cerenkov counting of the ^{32}P radioactivity in the histone bands indicates that insulin treatment of rat adipocytes results in a 97% increase in histone phosphorylation above basal level (Fig. 1*B*). The magnitude of insulin-stimulated histone V-S phosphorylation by HDM extracts tends to vary between 40% and 130% above control values; the average increase (>20 separate experiments) was 80%. In addition to stimulating the phosphorylation of histone, the hormone also enhances the ^{32}P-labeling of several HDM-associated proteins of 240, 140, 92, 67, and 47 kDa. The 92-kDa species is identified as the β subunit of the insulin receptor because it is specifically immunoprecipitated by patient-derived anti-insulin receptor antibodies (data not shown). The ^{32}P-labeled 240-, 140-, 67-, and 47-kDa proteins are not recognized by the insulin receptor antiserum and thus are unrelated to the receptor structure.

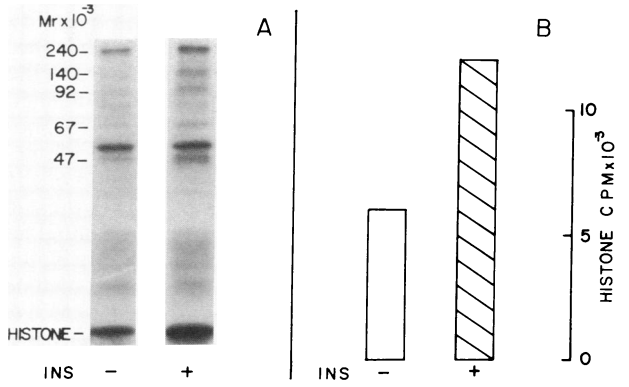

FIG. 1. Insulin stimulates HDM kinase activity. Aliquots (20 μl) of Triton X-100-solubilized HDM from control (INS −) and insulin-treated (INS +) adipocytes were incubated in the presence of [γ-^{32}P]ATP (100 μM) and histone (0.3 mg/ml) for 10 min at 22°C. The phosphorylation reaction was stopped by the addition of $NaDodSO_4$ and dithiothreitol. After the samples were electrophoresed in a $NaDodSO_4$/polyacrylamide gel, the proteins were fixed and the gel was autoradiographed. (*A*) Autoradiogram of the gel. (*B*) ^{32}P radioactivity associated with the histone bands as determined by Cerenkov counting.

Fig. 2 depicts the dose–response relationship of the adipocyte HDM-associated histone kinase activity and insulin receptor β-subunit phosphorylation to the insulin concentration. The insulin-sensitive membrane-associated histone kinase activity is highly sensitive to the hormone. Half-maximal and maximal activations of the HDM kinase activity are achieved at 0.6 nM and 10 nM insulin, respectively. In contrast, ≈10 times higher concentrations of insulin are required for similar responses in insulin receptor β-subunit phosphorylation (Fig. 2).

The hormone-mediated histone phosphorylation occurs on serine, threonine, and tyrosine residues (Fig. 3). These results indicate that serine/threonine and tyrosine kinase activities in adipocyte HDM are both activated following exposure of fat cells to insulin. The stimulatory effects of insulin on histone phosphorylation cannot be due to the action of the hormone on protein phosphatases, because the phosphorylation reaction is assayed in the presence of the phosphatase inhibitors NaF and sodium vanadate. No detectable dephosphorylation of ^{32}P-labeled proteins is observed under these conditions (data not shown).

Substrate-specificity studies reveal that the insulin-stimulated HDM kinase activity preferentially phosphorylates histones and the synthetic peptide Leu-Arg-Arg-Ala-Ser-Leu-Gly (Kemptide). The levels of histone and Kemptide phosphorylation in the insulin-treated group average at least 2-fold higher than those in the control. On the other hand, the hormone has no consistent effects on the phosphorylation of casein, ribosomal protein S6, ATP citrate lyase, phosvitin, glycogen synthase, and substrate peptides for multifunctional calmodulin-dependent protein kinase (20), casein kinase II (21), protein kinase C (22), and cGMP-dependent protein kinase (23). Furthermore, cAMP does not alter the stimulatory action of insulin on the HDM histone and Kemptide

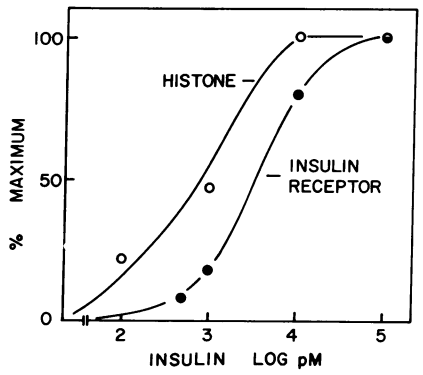

FIG. 2. Insulin dose–response of HDM histone kinase activity and insulin receptor β-subunit phosphorylation. Rat adipocytes were incubated in the absence or presence of insulin for 10 min at 37°C. The cells were homogenized and HDM were prepared by differential centrifugation. The HDM were solubilized in 1% Triton X-100 and then incubated with 100 μM [γ-^{32}P]ATP in the presence of histone. After electrophoresis, the proteins were fixed and the gel was autoradiographed. Histone phosphorylation (○) was quantitated by densitometric scanning of the autoradiogram. Insulin receptor β-subunit phosphorylation (●) was monitored in the same fashion following immunoprecipitation of the ^{32}P-labeled receptor from the HDM extracts with insulin receptor antiserum. Results are expressed as percentage of maximal stimulation in the presence of 10^{-7} M insulin.

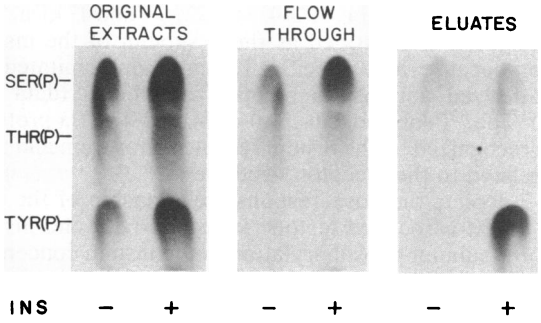

FIG. 3. Wheat germ agglutinin-agarose chromatography of control and insulin-stimulated histone kinase activities in adipocyte HDM. Triton X-100-solubilized HDM (140 μl) from control (INS −) and insulin-treated (INS +) rat adipocytes were centrifuged at 10,000 × *g* for 10 min at 4°C. The supernatants were recycled twice through a wheat germ agglutinin-agarose column (25 μl). The column was washed three times each with 0.2 ml of buffer A containing 1% Triton X-100. The adsorbed materials were eluted with 0.1 ml of 0.3 M *N*-acetylglucosamine in the same buffer. The histone kinase activities in 20 μl of the original HDM detergent extracts, the flow-through, and the eluted fractions were assayed. The samples were electrophoresed in $NaDodSO_4$/10% polyacrylamide gel. The histone bands were located and excised. The phospho amino acid contents in the ^{32}P-labeled histone were analyzed.

kinase activities, although the basal levels of phosphorylation are elevated.

Resolution of Insulin-Stimulated Serine and Tyrosine Kinase Activities by Wheat Germ Agglutinin-Agarose Chromatography. The insulin-activated serine and tyrosine kinase activities can be readily separated by the lectin column (Fig. 3). The hormone-sensitive serine kinase is not adsorbed by the immobilized lectin, whereas the tyrosine kinase activity is retained and can be subsequently eluted with 0.3 M *N*-acetylglucosamine. These results indicate that the insulin-sensitive serine and tyrosine phosphorylation of histone by adipocyte HDM is catalyzed by at least two distinct kinases. Furthermore, the insulin-stimulated serine kinase may not be a glycoprotein, in view of its lack of interaction with the lectin column. On the other hand, the hormone-activated tyrosine kinase is a sugar-containing enzyme, as evidenced by its adsorption to the wheat germ agglutinin-agarose column.

To determine the relative proportion of insulin-stimulated serine/threonine and tyrosine kinase activities in the adipocyte microsomal extracts, the magnitudes of histone phosphorylation by the lectin-agarose-resolved HDM detergent fractions were measured. As shown in Fig. 4*A*, the serine/threonine kinase activity in the flow-through accounts for 60% of the total insulin-stimulated histone phosphorylating activity in the HDM extracts. The remaining 40% represents hormone-sensitive tyrosine kinase activity that is adsorbed by the wheat germ agglutinin-agarose column.

Kinetic Properties of HDM Serine Kinase. The kinetic properties of the insulin-stimulated serine kinase activity, freed from the contaminating tyrosine kinase activity by wheat germ agglutinin-agarose chromatography, were investigated. As shown in Fig. 4*B*, both the basal and the insulin-stimulated ^{32}P-labeling of histone by this material increase progressively with reaction time. The reaction is also dependent on the concentration of HDM extracts and substrate (Fig. 4 *C* and *D*). However, the dependencies of HDM serine kinase activity on these three parameters are not strictly linear. The nonlinearity in kinase activities with respect to time and enzyme concentration is most likely due to the different extents of ATP hydrolysis when these two conditions are varied. On the other hand, the leveling of the histone concentration curve may be attributed to substrate saturation of the HDM serine kinase.

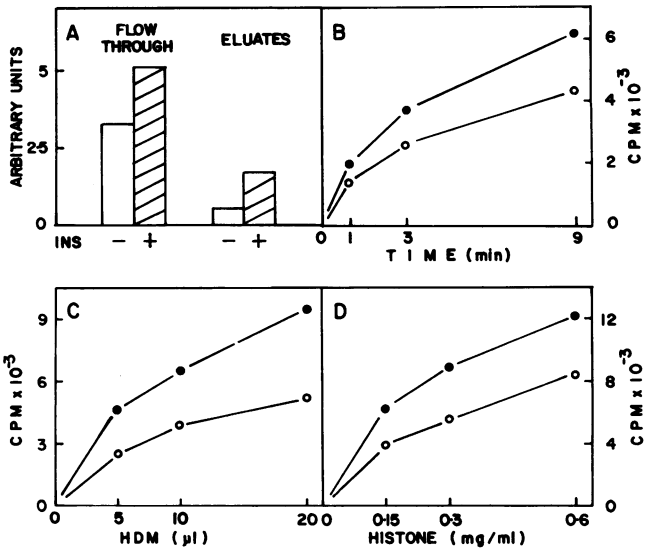

FIG. 4. Relative abundance and kinetic properties of HDM serine kinase activity. Aliquots (20 μl) of the flow-through and eluted fractions derived from wheat germ agglutinin-agarose chromatography of HDM detergent extracts were assayed for insulin-stimulated serine and tyrosine kinase activities using histone type V-S as substrate. Histone phosphorylation was quantitated by densitometric scanning of autoradiograms. Total kinase activity in the aliquots was calculated after correction for the total volume in each fraction. The final results from one representative experiment are shown in *A*. Similar results were obtained in replicate experiments. Insulin-sensitive HDM serine kinase activity in fractions that did not adsorb to lectin-agarose was assayed for dependence on time (*B*), enzyme concentration (*C*), and substrate concentrations (*D*). For the enzyme-concentration experiments, various volumes (5–20 μl) of HDM extract were used; the total reaction volume was maintained at 33 μl. The HDM serine kinase activity under each condition was monitored using histone type V-S and [γ-^{32}P]ATP. Histone phosphorylation was determined by Cerenkov counting of the ^{32}P-labeled histone band after electrophoresis of the samples. Each experimental point represents the mean of two determinations minus the appropriate background value. Open symbols, control. Closed symbols, plus insulin.

Adsorption of Insulin-Stimulated Histone Kinase Activity to Anti-Phosphotyrosine Antibodies. In an attempt to define the nature of the stimulatory effects of insulin on the HDM-associated histone kinase(s), we investigated the possibility that the activation process may involve tyrosine phosphorylation of the membrane bound kinase(s) in response to the hormone. If this hypothesis is correct, the insulin-stimulated histone kinase activity may be adsorbed by anti-phosphotyrosine antibodies due to the presence of this phospho amino acid in the enzyme(s). As shown in Fig. 5, a portion of the insulin-stimulated serine and tyrosine kinase activities in HDM is adsorbed by the anti-phosphotyrosine immunoglobulin immobilized on agarose and can be subsequently eluted with 10 mM *p*-nitrophenyl phosphate. The adsorbed histone kinase activities represent ≈22% of the total insulin-sensitive kinase activities in HDM.

Phospho amino acid analyses of the ^{32}P-labeled histone indicate that all the antibody-bound insulin-activated serine kinase activity and 80% of the hormone-sensitive tyrosine kinase activity can be eluted with *p*-nitrophenyl phosphate. Only phosphotyrosine (1 mM) is effective in blocking the adsorption of membrane-associated kinase activity by the antibody-agarose; phosphoserine and phosphothreonine at the same concentration are ineffective (Table 1). It is clear that the inclusion of phosphotyrosine in the adsorption buffer significantly lowers both the basal and the insulin-stimulated histone phosphorylation that is bound by the matrix (by 50%

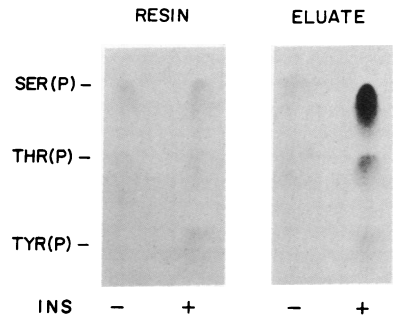

FIG. 5. Adsorption of HDM histone kinase activities to anti-phosphotyrosine antibodies. Detergent-solubilized HDM (70 μl) from control (INS −) and insulin-treated (INS +) fat cells were mixed with 10 μl of anti-phosphotyrosine antibody-agarose at 4°C for 2 hr. The immunoglobulin-agarose was sedimented at 600 × *g* and washed three times with buffer A containing 1% Triton X-100. Elution of adsorbed materials was effected by incubating the resin in 40 μl of buffer A/1% Triton/10 mM *p*-nitrophenyl phosphate at 4°C for 10 min. The samples were centrifuged (10,000 × *g*) and 20 μl of each supernatant (*p*-nitrophenyl phosphate eluate) was removed. The remaining resin was washed twice and finally suspended in 10 μl of buffer A with 1% Triton X-100. The histone kinase activities in the fraction eluted with *p*-nitrophenyl phosphate and in the remaining resin were assayed using 20-μl samples. Phospho amino acid contents of histone ^{32}P-labeled by each fraction were analyzed.

and 60%, respectively). The presence of phosphotyrosine almost completely blocks the adsorption of the insulin-sensitive portion of histone kinase activity to the immobilized immunoglobulin. Phospho amino acid analysis of the ^{32}P-labeled histone shows that the competitive inhibition of phosphotyrosine is directed at both the serine and the tyrosine kinase activities (data not shown). These results suggest that a significant portion of the insulin-sensitive HDM-associated serine/threonine protein kinase molecules contain phosphotyrosine residues.

DISCUSSION

The results show that a serine/threonine kinase associated with a specific cellular membrane fraction is rapidly activated after exposure of rat adipocytes to insulin. In addition, an insulin-mediated increase in tyrosine kinase activity is detected in the same membrane preparation. The membrane-associated serine/threonine kinase activity is highly sensitive to insulin (ED_{50} = 0.6 nM) and is markedly stimulated when only a small fraction of the insulin receptor kinase is activated by the hormone (Fig. 2). The stimulatory action of insulin on the HDM serine kinase can only be detected in membranes from hormone-treated adipocytes. Direct addition of insulin to isolated membrane preparations fails to activate the histone serine kinase activity, although the hormone readily enhances the tyrosine phosphorylation of histone under these conditions (data not shown). The reason for this discrepancy is unclear. As discussed below, the histone tyrosine kinase activity may be contributed by the insulin receptor, which can be directly activated by insulin in the membrane detergent extracts. In contrast, the insulin-dependent stimulation of histone serine kinase activity may require other hormone-sensitive processes that can only occur in intact cells. The insulin-dependent serine kinase activity reported in the present studies in HDM appears to be distinct from the ribosomal protein S6 kinase, which is activated by a number of hormones. This conclusion is based on the following: (*i*) the insulin-sensitive HDM serine kinase does not efficiently phosphorylate exogenous S6 protein under conditions in which the phosphorylation of histone is markedly stimulated; (*ii*) the S6 kinase does not phosphorylate histone (5, 24); (*iii*) in the phosphorylation reaction buffer, we include a high concentration of NaF (100 mM), which has been shown to completely abolish the insulin stimulation of S6 kinase activity (24, 25). In light of this evidence it is unlikely that the insulin-stimulated histone serine kinase in adipocyte HDM is related to the S6 kinase. Furthermore, the HDM serine kinase is distinct from kinases that phosphorylate ATP citrate lyase, glycogen synthase, casein, and phosvitin and from multifunctional calmodulin-dependent, cGMP-dependent, cAMP-dependent, or Ca^{2+}/phospholipid-dependent protein kinases. Thus, our data suggest that the HDM-associated serine/threonine kinase is an insulin-sensitive enzyme not described previously.

Table 1. Specificity of adsorption of HDM histone kinase by anti-phosphotyrosine antibodies

Adsorption inhibitor	Histone phosphorylation, arbitrary units		
	Control	Insulin	Increase due to insulin
None	14.6	20.6	6.0
Phosphotyrosine	7.2	8.2	1.0
Phosphoserine	15.2	20.3	5.1
Phosphothreonine	14.1	20.2	6.1

Aliquots (20 μl) of HDM detergent extracts from control and insulin-treated adipocytes were mixed with 10 μl of immobilized anti-phosphotyrosine antibodies in the absence or presence (1 mM) of phosphotyrosine, phosphoserine, or phosphothreonine at 4°C for 2 hr. The agarose was washed three times and then the adsorbed materials were eluted with 40 μl of buffer A/1% Triton X-100/10 mM nitrophenylphosphate. Histone kinase activities in the eluates (20 μl) were monitored according to the procedure described in the legend to Fig. 1. Histone phosphorylation was quantitated by densitometric scanning.

The identity of the insulin-sensitive tyrosine kinase in HDM has yet to be established, although all or part of this tyrosine kinase activity may be contributed by the insulin receptor. Previous studies (26, 27) indicated that insulin receptors derived from insulin-treated cells exhibit a 20-fold increase in tyrosine kinase activity toward exogenous histone and that this increase is associated with an increase in phosphotyrosine content in the receptor β subunit. As is expected for the insulin receptor, the insulin-sensitive tyrosine kinase activity identified in this study is a glycoprotein, as evidenced by its adsorption to wheat germ agglutinin-agarose (Fig. 3). Nevertheless, the possibility still exists that another tyrosine kinase distinct from the insulin receptor kinase is activated in HDM in response to insulin.

To examine the possibility that the HDM histone kinases are activated following hormone-induced tyrosine phosphorylation of the enzymes, we used monoclonal antibodies generated against phosphotyrosine. Fig. 5 and Table 1 show that 22% of insulin-activated histone kinase activities can be specifically adsorbed by the monoclonal antibody preparation and can be subsequently eluted with *p*-nitrophenyl phosphate. Most important, the eluted insulin-sensitive kinase activities are capable of phosphorylating histone on serine as well as tyrosine residues. These results suggest that the insulin-activated serine kinase that is recognized by the antibody-agarose may contain more phosphotyrosine than the kinase derived from control cells. However, adsorption of insulin-stimulated serine kinase activity by the anti-phosphotyrosine antibodies is incomplete: only 20–25% of this activity binds to this resin. It is possible that this kinase activity is associated with tyrosine phosphorylation at a high stoichiometry but that the monoclonal antibody preparation may not bind the tyrosine-phosphorylated kinase efficiently, because of either steric or charge effects around the phosphorylation site. Alternatively, it is possible that not more than 20–25% of the insulin-stimulated kinase molecules are phosphorylat-

ed on tyrosine. The present study provides strong support for the hypothesis that at least a significant fraction of the insulin-stimulated serine kinase in HDM is phosphorylated on tyrosine residues.

The adsorption of insulin-stimulated serine kinase activity to anti-phosphotyrosine antibodies raises the possibility that the hormone-sensitive kinase may be activated by tyrosine phosphorylation. However, the activity of the insulin-sensitive serine kinase in our studies is monitored in the absence of any information on the absolute amount of kinase immobilized by the anti-phosphotyrosine antibody preparation. It is thus not possible to infer that insulin actually increases the number of HDM serine kinase molecules that are tyrosine-phosphorylated. It is also possible that the insulin-stimulated serine kinase activity in HDM may be tyrosine-phosphorylated in the absence of insulin, but that the stimulatory action of the hormone may be effected through other modifications of the enzyme. Therefore, purification of the insulin-sensitive serine kinase from adipocyte HDM will be necessary to further evaluate possible modulation in activity of this serine kinase by tyrosine phosphorylation.

We thank Dr. William B. Benjamin for the gift of ATP citrate lyase. The meticulous secretarial work of Karen Donahue and Mary Halley is acknowledged with thanks. This work is supported by Grants AM30648 and AM35399 by the National Institutes of Health.

1. Cohen, P. (1982) *Nature (London)* **296,** 613–619.
2. Czech, M. P., Massague, J., Seals, J. R. & Yu, K. T. (1984) in *Biochemical Actions of Hormones*, ed. Litwack, G. (Academic, New York), Vol. 11, pp. 93–125.
3. Smith, C. J., Rubin, C. S. & Rosen, O. M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2641–2645.
4. Thomas, G., Martin-Perez, J., Siegmann, M. & Otto, A. (1982) *Cell* **30,** 235–242.
5. Cobb, M. H. & Rosen, O. M. (1983) *J. Biol. Chem.* **258,** 12472–12481.
6. Maller, J. L., Pike, L. J., Freidenberg, G. R., Cordera, R., Stith, B. J., Olefsky, J. M. & Krebs, E. G. (1986) *Nature (London)* **320,** 459–460.
7. Avruch, J., Nemenoff, R. A., Pierce, M., Kwok, Y. C. & Blackshear, P. J. (1985) in *Molecular Basis of Insulin Action*, ed. Czech, M. P. (Plenum, New York), pp. 263–296.
8. Kasuga, M., Zick, Y., Blithe, D. L., Karlsson, F. A., Haring, H. U. & Kahn, C. R. (1982) *J. Biol. Chem.* **257,** 9891–9894.
9. Kasuga, M., Fujita-Yamaguchi, Y., Blithe, D. L. & Kahn, C. R. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 2137–2141.
10. Ullrich, A., Bell, J. R., Chen, E. Y., Herrera, R., Petruzzelli, L. M., Dull, T. J., Gray, A., Coussens, L., Liao, Y. C., Trubokawa, M., Mason, A., Seeburg, O. H., Grunfeld, C., Rosen, O. M. & Ramachandran, J. (1985) *Nature (London)* **313,** 756–761.
11. 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.
12. Takayama, S., White, M. F., Lauris, V. & Kahn, C. R. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 7797–7801.
13. Morgan, D. O., Ho, L., Korn, L. J. & Roth, R. A. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 328–332.
14. Perisic, O. & Traugh, J. A. (1983) *J. Biol. Chem.* **258,** 9589–9592.
15. Rodbell, M. (1964) *J. Biol. Chem.* **239,** 375–380.
16. McKeel, D. W. & Jarett, L. (1970) *J. Biol. Chem.* **44,** 417–432.
17. Oka, Y., Mottola, C., Oppenheimer, C. L. & Czech, M. P. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4028–4032.
18. Klarlund, J. K. (1985) *Cell* **41,** 707–717.
19. Yu, K. T. & Czech, M. P. (1984) *J. Biol. Chem.* **259,** 5277–5286.
20. Pearson, R. B., Woodgett, J. R., Cohen, P. & Kemp, B. E. (1985) *J. Biol. Chem.* **260,** 14471–14476.
21. Kuenzel, E. A. & Krebs, E. G. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 737–741.
22. Hunter, T., Ling, N. & Cooper, J. A. (1984) *Nature (London)* **311,** 480–483.
23. Glass, D. B. & Krebs, E. G. (1982) *J. Biol. Chem.* **257,** 1196–1200.
24. Tabarini, D., Heinrich, J. & Rosen, O. M. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 4369–4373.
25. Novak-Hofer, I. & Thomas, G. (1985) *J. Biol. Chem.* **260,** 10314–10319.
26. Klein, H. H., Freidenberg, G. R., Kladde, M. & Olefsky, J. M. (1986) *J. Biol. Chem.* **261,** 4691–4697.
27. Yu, K. T. & Czech, M. P. (1986) *J. Biol. Chem.* **261,** 4715–4722.