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Phosphorylation of Glycolytic and Gluconeogenic Enzymes by the Insulin Receptor Kinase

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Various glycolytic and gluconeogenic enzymes were tested as substrates for the insulin receptor kinase. Phosphofructokinase and phosphoglycerate mutase were found to be the best substrates. Phosphorylation of these enzymes was rapid, stimulated 2- to 6-fold by 10^{-7} M insulin and occurred exclusively on tyrosine residues. Enolase, fructose 1,6-bisphosphatase, lactate dehydrogenases in decreasing order, were also subject to insulin-stimulated phosphorylation but to a smaller extent than that for phosphofructokinase or phosphoglycerate mutase.

The phosphorylation of phosphofructokinase was studied most extensively since phosphofructokinase is known to catalyze a rate-limiting step in glycolosis. The apparent Km of the insulin receptor for phosphofructokinase was 0.1 μM, which is within the physiologic range of concentration of this enzyme in most cells. Tyrosine phosphorylation of phosphofructokinase paralleled autophosphorylation of the β-subunit of the insulin receptor with respect to time course, insulin dose response (half maximal effect between 10^{-9} and 10^{-8} M insulin), and cation requirement ($Mn^{2+} > Mg^{2+} >> Ca^{2+}$). Further study will be required to determine whether the tyrosine phosphorylation of phosphofructokinase plays a role in insulin-stimulated increases in glycolytic flux.

Key words: phosphorylation, insulin receptor, tyrosine kinase, phosphofructokinase, glycolysis

Insulin initiates its action in target cells by binding to a specific receptor on the plasma membrane. Recent evidence has shown that the insulin receptor possesses a tyrosine kinase activity that catalyzes autophosphorylation of the β-subunit and phosphorylation of exogenous substrates [1–13]. The tyrosine kinase activity is stimulated by insulin and may play a role in post-receptor transfer of the insulin signal.

Tyrosine-specific kinases have been shown to be associated with the oncogene products of Rous sarcoma virus (SRC), Fujinami sarcoma virus (FPS), Abelson

Abbreviations used: PFK, phosphofructokinase; PGM phosphoglycerate mutase; $pp60^{v\text{-}src}$, gene product of Rous sarcoma virus; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; $NaDodSO_4$, sodium dodecyl sulphate; PAGE, polyacrylamide gel electrophoresis.

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murine leukemia virus (ABL), and Snyder-Theile feline sarcoma virus (FES) [for review, see 14]. Cells transformed by these viruses contain elevated levels of phosphotyrosine on a number of proteins. Three proteins found to have phosphotyrosine in these cells are the glycolytic enzymes PGM, enolase, and lactate dehydrogenase [15–18]. The extent of phosphorylation of these enzymes varies according to the transforming virus. Viruses which do not encode tyrosine kinases such as SV40, polyoma virus, McDonough feline sarcoma virus (FMS), or Moloney murine sarcoma virus (MOS) do not enhance the phosphotyrosine content of PGM [16]. Since insulin is known to increase flux through the glycolytic pathway [19,20] and the insulin receptor is a tyrosine kinase, in the present work we have examined a number of glycolytic enzymes as substrates for the insulin receptor tyrosine kinase in vitro.

MATERIALS AND METHODS

Phosphofructokinase (PFK) purified from rabbit skeletal muscle was the kind gift of Dr. R.G. Kemp (University of Chicago) or obtained from Sigma (St. Louis, MO). Both sources gave similar results. Other glycolytic enzymes purified from skeletal muscle and fructose 1,6-bisphosphatase purified from liver were purchased from Sigma. All glycolytic and gluconeogenic enzymes were desalted by dialysis at 5°C for 14 hr against 500 volumes of 50 mM HEPES (pH 7.4) prior to use in phosphorylation assays. Partially purified insulin receptor was prepared from a hepatoma cell line (Fao) by affinity chromatography on wheat germ agglutinin as previously described [21].

[γ $-^{32}$P]ATP was from New England Nuclear (Boston, MA), and reagents for SDS-PAGE were from Bio-Rad (Richmond, CA). Porcine insulin was purchased from Elanco (Indianapolis, IN). Other biochemicals and reagents were of the purest grade available from Fisher (Springfield, NJ) or Sigma.

Phosphorylation Assay

Partially purified insulin receptor (3–4 μg total protein) was incubated for 45–60 min at 22°C with or without insulin at the concentrations indicated in 30–40 μl of 50 mM HEPES (pH 7.4), 0.1% Triton X-100 containing $MgCl_2$ (5 mM), $MnCl_2$ (5 mM), or $CaCl_2$ (0.5 mM) as detailed in the text. The glycolytic/gluconeogenic enzymes were added at the concentrations shown, and the phosphorylation reaction was initiated with [γ $-^{32}$P]ATP (25–250 μ M, 6,000–12,000 cpm/pmol). Incubation was terminated after the times indicated by the addition of Laemmli sample buffer [22] containing dithiothreitol (100 mM). Alternatively, reactions were stopped by the addition of 400 μl of 10% trichloroacetic acid and 10 μg cytochrome C as protein carrier. Protein was sedimented by centrifugation (8,000g, 3 min). The pellets were washed with diethyl ether and finally resuspended in sample buffer. Phosphoproteins were separated by $NaDodSO_4$ polyacrylamide gel electrophoresis according to the method of Laemmli [22] and analyzed by autoradiography. Gels were exposed to Kodak X-Omat AR film overnight at −80°C using Cronex lightening plus intensifying screen. ^{32}P incorporated into proteins was assessed by liquid scintillation counting of appropriate gel pieces or by densitometric scanning of autoradiograms. Results are mean ± standard error of the mean for the number of observations (n) given in parentheses.

Phosphoamino Acid Analysis

Phosphopeptides were digested with 100 μg trypsin (in 2 ml of 50 mM H_4HCO_3, pH 8) and then hydrolysed with 6 N HCl for 2 hr at 100°C [see ref. 23]. The phosphoamino acids were separated by high-voltage electrophoresis on thin-layer plates (Avicil, Analtech, 250) by using a solution of H_2O: acetic acid: pyridine (189:10:1) at pH 3.5. Phosphotyrosine, phosphoserine, and phosphothreonine standards were added to all radioactive samples and identified by reaction with ninhydrin.

RESULTS

Phosphorylation of Glycolytic and Gluconeongenic Enzymes by the Insulin Receptor

The enzymes, phosphofructokinase (PFK, EC 2.7.1.11), and phosphoglycerate mutase (PGM, EC 5.4.2.1), enolase (EC 4.2.1.11), lactate dehydrogenase (EC 1.1.1.27), and fructose 1,6-bisphosphatase (EC 3.1.3.11) were phosphorylated when incubated with insulin receptor partially purified from rat Fao hepatoma cells and [$\gamma-^{32}$P]ATP (Fig. 1). Under these conditions, ^{32}P-incorporation into PFK and PGM was most evident and was stimulated by 10^{-7} M insulin 340 $\pm$ 40% (n = 8) and 300 $\pm$ 75% (n = 4), respectively. These insulin effects were highly reproducible between different insulin receptor preparations and different preparations of pure enzymes. Under identical conditions, autophosphorylation of the β-subunit of the insulin receptor (M_r 95,000) was stimulated 4.6 $\pm$ 0.4 (n = 6) fold by 10^{-7} M insulin and was unaffected by the presence of glycolytic and gluconeogenic enzymes in the phosphorylation assay.

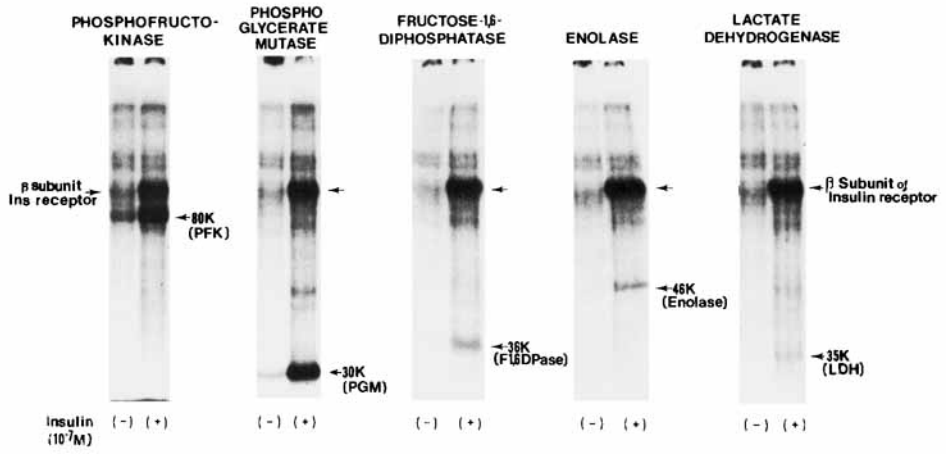

Fig. 1. Phosphorylation of glycolytic/gluconeogenic enzymes by the insulin receptor in vitro. Insulin receptor partially purified from Fao hepatoma cells (2.5 μg protein) was pre-incubated at 22°C with 5 mM $MnCl_2$ and 5 mM $MgCl_2$ in the absence (−) or presence (+) of 10^{-7} M insulin. After 1 hr, enzymes were added as follows: skeletal muscle phosphofructokinase (7 μg); phosphoglycerate mutase (5 μg); fructose-1,6-bisphosphatase (3 μg); enolase (2 μg); lactate dehydrogenase (3 μg). The phosphorylation reaction was initiated with [γ^{32}P]ATP (40 μM final concentration). The reaction was terminated after 10 min, and phosphoproteins were analyzed by electrophoresis on 7.5% polyacrylamide gels and with autoradiography.

When expressed as mole phosphate incorporated per mole of enzyme, the insulin-stimulated phosphorylation of lactate dehydrogenase, enolase, and fructose 1,6-bisphosphatase were about 5% to 20% of that of PFK. No ^{32}P was incorporated into any of the glycolytic and gluconeogenic enzymes when incubated under identical conditions in the absence of insulin receptor with or without insulin (data not shown).

Phosphoamino acid analysis showed that the insulin-stimulated phosphorylation of the bands of 95KDa (β-subunit of insulin receptor), 80KDa (PFK) and 32KDa (PGM) occurred exclusively on tyrosine residues (Fig. 2). A small degree of phosphate associated with PFK was also located on serine and threonine residues, but levels of these phosphoamino acids were unaffected by insulin. A cAMP-dependent phosphorylation site of PFK on serine residues has been described previously [24,25]. Thus, the small degree of serine phosphorylation observed in the present study may be the result of very low levels of contaminating cAMP-dependent protein kinase activity.

Since PFK is a rate-limiting enzyme in glycolysis [26–28] and appeared to be the best substrate for the insulin receptor kinase, further studies were conducted characterizing the phosphorylation of PFK. Limited studies were also carried out investigating the phosphorylation of PGM.

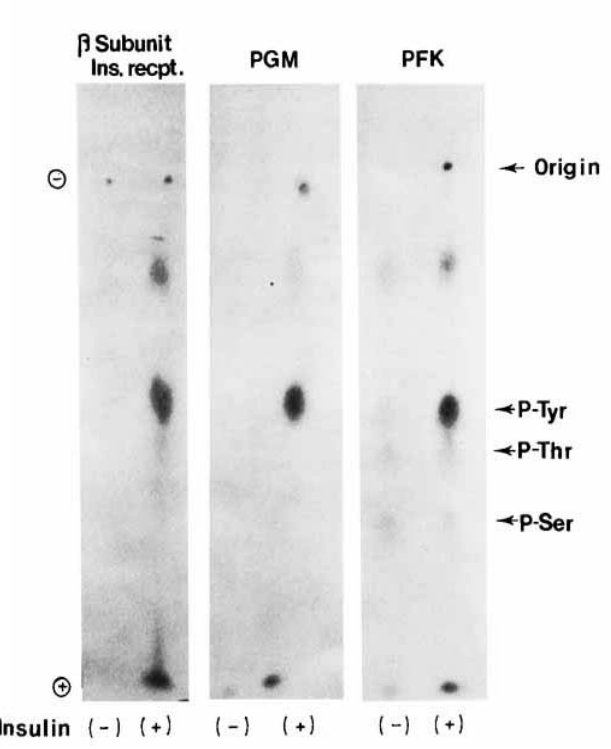

Fig. 2. Phosphoamino acid analysis. The phosphoprotein bands of the insulin receptor (95KDa, β-subunit), PFK (80KDa), and PGM (30KDa) obtained in Figure 1 were subjected to acid hydrolysis in 6N HCl. Phosphoamino acids were separated by electrophoresis on thin layer plates. The mobility of phosphoserine, phosphothreonine, and phosphotyrosine, determined by the addition of standards to all radioactive samples, is shown.

Time Course of Phosphorylation of Phosphofructokinase and Phosphoglycerate Mutase

^{32}P-incorporation into PFK or PGM exhibited a similar time course to that of the β-subunit of the insulin receptor (Fig. 3A,B). Phosphorylation of both glycolytic enzymes and the β-subunit of the insulin receptor was stimulated by 10^{-7} M insulin 2- to 4-fold. Fifty percent of maximum ^{32}P-incorporation into PFK occurred after approximately 5 min at 22°C. The ^{32}P content of both PFK and the insulin receptor decreased at 60 min, probably as a result of some phosphatase activity in the wheat germ agglutinin preparation of insulin receptor [29].

Insulin-Dose Response for Stimulation of Phosphorylation

The effect of insulin concentration on the phosphorylation of PFK and the insulin receptor is shown in Fig. 4. In the absence of insulin, incorporation of ^{32}P into both proteins was low, and incorporation greatly increased in parallel with increasing insulin concentration up to 10^{-7} M insulin. Further increases in insulin concentration were without effect. Half maximal stimulation of phosphorylation of PFK and the β-subunit of insulin receptor occurred between 10^{-9} and 10^{-8} M insulin under the conditions studied (Fig. 4B).

Effect of Cations on Insulin-Mediated Phosphorylation of Phosphofructokinase

To further establish involvement of the insulin receptor kinase, the ion requirement for phosphorylation of the glycolytic enzymes was investigated. Divalent metal ions enhance autophosphorylation of the insulin receptor in the order $Mn^{2+} > Mg^{2+} >> Ca^{2+}$ [30]. As shown in Figure 5, the effect of these cations on the phosphorylation of PFK was similar, ie, at equal concentration (5 mM), Mn^{2+} was a more potent cation than Mg^{2+}. Ca^{2+} was without effect. The presence of Mn^{2+} and Mg^{2+} together did not enhance phosphorylation of PFK above that by Mn^{2+} alone (data not shown).

Effect of Phosphofructokinase and ATP Concentration

When the concentration of all other components remained unchanged, ^{32}P incorporated into PFK showed a hyperbolic relationship with respect to PFK concentration (Fig. 6). The apparent Km for PFK in the absence and presence of insulin was approximately 0.1 μM tetramers. Phosphorylation of PFK was maximal at 0.4 μM PFK tetramers. Similarly, tyrosine phosphorylation of PGM was also detectable at submicromolar concentrations of the enzyme (ie, 0.1 μM dimers; data not shown). In each case, ^{32}P incorporated into PFK or PGM in the presence of insulin was significantly higher than in its absence at every enzyme concentration tested.

The effect of ATP concentration on the phosphorylation of PFK and the insulin receptor is shown in Figure 7a. In the presence of 5 mM Mn^{2+}, maximum ^{32}P-incorporation into PFK in basal and insulin-stimulated states occurred at 100 μM ATP with apparent Km of 30–35 μM. The dependence of insulin receptor phosphorylation on ATP concentration in the absence of insulin was similar (Fig. 7b; apparent Km = 38 μM). However, with insulin present, the apparent Km was 11 μM. A similarly low Km value for ATP (19 μM) was observed in Reference 21 in the presence of insulin. This may mean that insulin increases the affinity of the receptor for ATP for

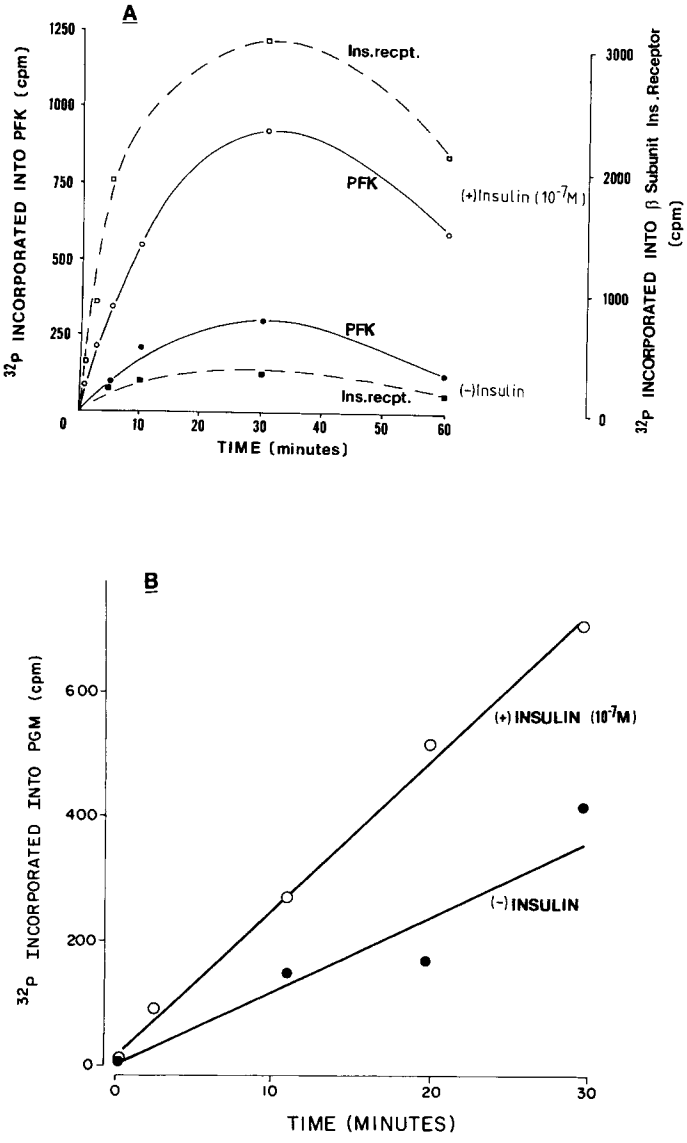

Fig. 3. Time course for the phosphorylation of (A) phosphofructokinase or (B) phosphoglycerate mutase by the insulin receptor kinase. Insulin receptor (3.5 μg protein) was pre-incubated for 1 hr with 5 mM $MnCl_2$ and 5 mM $MgCl_2$ in the absence (closed symbols) or presence (open symbols) of 10^{-7} M insulin as in Figure 1. The phosphorylation was carried out with (A) 7 μg purified PFK and (B) 10 μg purified PGM. Assays (A) and (B) were conducted using the same preparation of insulin receptor. The reaction was initiated with [γ $-^{32}$P]ATP (30 μM final concentration) and allowed to proceed for the times indicated. ^{32}P incorporated into the protein bands of 95KDa (β-subunit of insulin receptor), 80KDa (PFK), and 30KDa (PGM), separated by SDS gel electrophoresis, was assessed by liquid scintillation counting of appropriate gel slices.

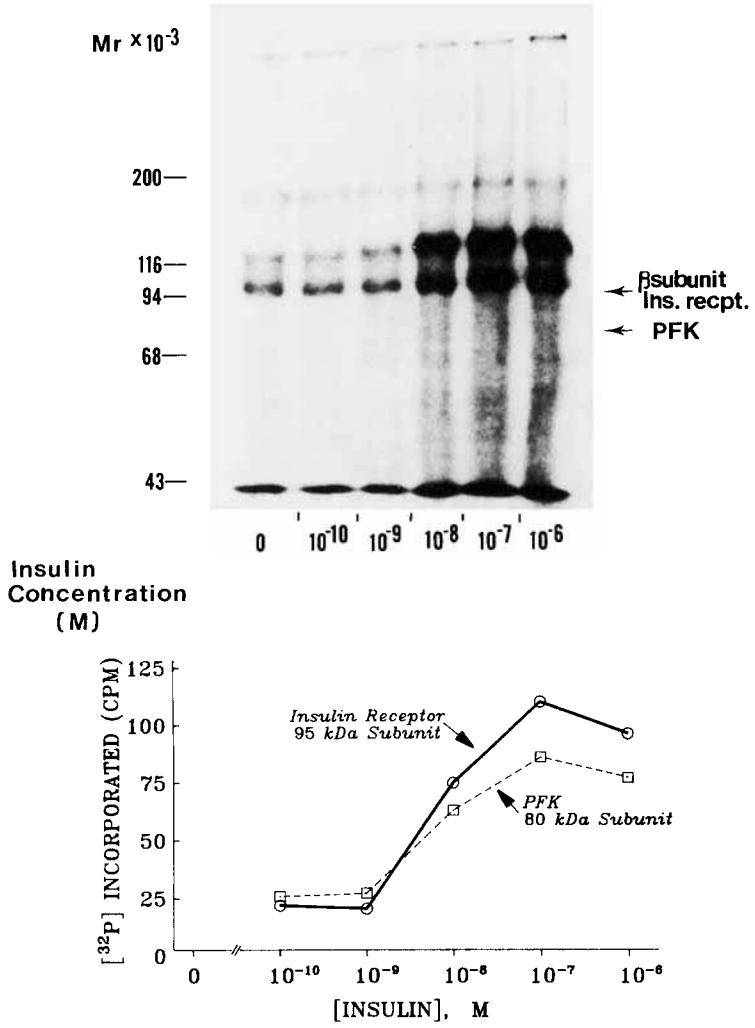

Fig. 4. Insulin-dose response for the stimulation of phosphorylation of phosphofructokinase. Partially purified insulin receptor (3 μg protein) was pre-incubated at 22°C with 5 mM $MnCl_2$ in the presence of insulin at the concentrations indicated. After 1 hr, 3 μg purified PFK was added and the phosphorylation reaction initiated by the addition of [γ $-^{32}$P]ATP (100 μM final concentration). The reaction was terminated after a further 15 min, and proteins were analyzed by SDS gel electrophoresis and radioautography. ^{32}P incorporated into PFK and β-subunit of insulin receptor was assessed by liquid scintillation counting of gel slices (Fig. 4B).

autophosphorylation but not for the phosphorylation of an exogenous substrate such as PFK.

DISCUSSION

The recent demonstration that the insulin receptor is an insulin-stimulated protein kinase [7, 9–12] and the fact that insulin alters the phosphorylation state of a variety of cellular enzymes [for review, see ref. 31] suggest a cascade of protein

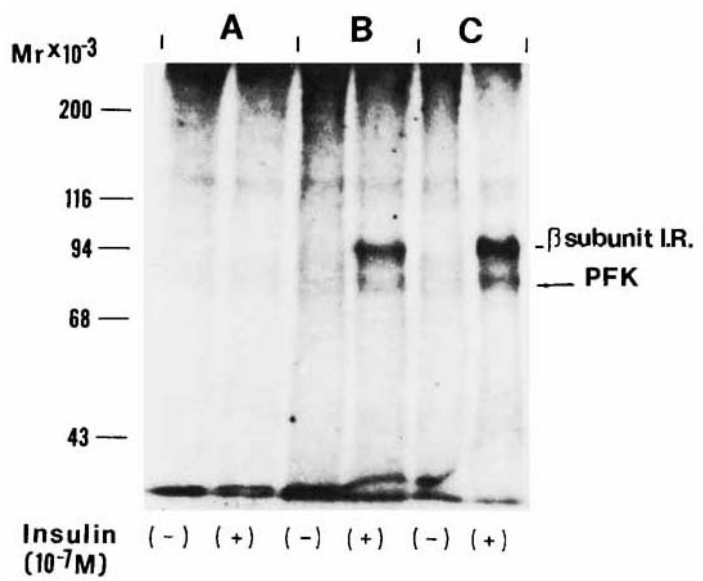

Fig. 5. Effect of cations of insulin-mediated phosphorylation of phosphofructokinase. Purified receptor (2 μg protein) was incubated for 1 hr in the absence (−) or presence (+) of 10^{-7} M insulin with (A) 0.5 mM $CaCl_2$, (B) 5 mM $MgCl_2$, (C) 5 mM $MnCl_2$. The phosphorylation reaction was then conducted for 20 min with 3 μg PFK and [$\gamma-^{32}$P]ATP (30 μM final concentration). Phosphoproteins were separated by SDS gel electrophoresis and analyzed by autoradiography.

phosphorylation/dephosphorylation as a possible mechanism by which insulin mediates its action. In the present work, we have shown that several glycolytic enzymes, as well as fructose 1,6-bisphosphatase, act as substrates for the insulin receptor kinase in vitro. When expressed as mole phosphate/mole enzyme subunit, PFK was the best substrate, followed in order of decreasing effectiveness by PGM, enolase, fructose 1,6-bisphosphatase, and lactate dehydrogenase. Phosphorylation of these enzymes was greatly stimulated by insulin and, where tested, occurred almost exclusively on tyrosine residues. Further study using PFK and PGM showed that phosphorylation of these enzymes paralleled closely the autophosphorylation of the β-subunit of the insulin receptor with respect to time course, and insulin-dose response. Furthermore, these phosphorylation reactions required the presence of divalent metal ions with $Mn^{2+} > Mg^{2+} >> Ca^{2+}$ similar to the cation requirement of the insulin receptor for autophosphorylation. The tyrosine kinases catalyzing phosphorylation of glycolytic enzymes and the β-subunit of the insulin receptor are therefore likely to be the same.

Presk et al [32] have demonstrated enhanced phosphorylation of the regulatory glycolytic enzyme pyruvate kinase (M_2-type) in RSV-transformed chicken embryo cells and on incubation of the enzyme with purified $pp60^{v\text{-}src}$. Furthermore, phosphoglycerate mutase, lactate dehydrogenase, and enolase are phosphorylated on tyrosine in cells transformed by Rous sarcoma virus, avian sarcoma virus, Fujinami sarcoma virus, and Snyder-Theilen feline sarcoma virus [15–17]. No increase in the phosphotyrosine content of these enzymes is observed on transformation of cells with viruses which do not encode tyrosine kinases. Thus, glycolytic enzymes are targets for tyrosine kinases in vivo. The sites of phosphorylation of lactate dehydrogenase

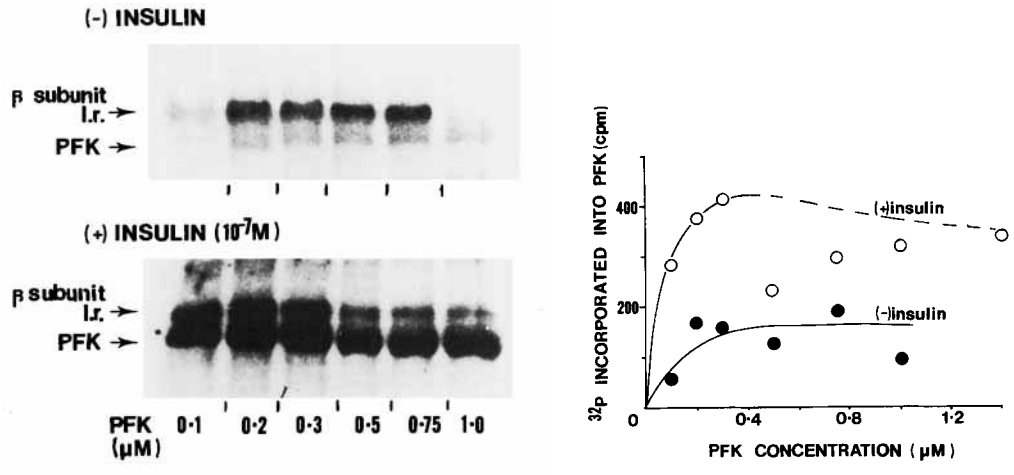

Fig. 6. Effect of phosphofructokinase concentration. Insulin receptor (3.5 μg protein) was preincubated in the absence or presence of insulin (10^{-7} M) as in Figure 1. The phosphorylation reaction was carried out for 15 min with [$\gamma-^{32}$P]ATP (100 μM final concentration) and PFK at the concentrations indicated. ^{32}P incorporated into the PFK band (80KDa) separated by SDS/gel electrophoresis was determined as in Figure 3.

and enolase in RSV-transformed cells have been identified as single specific tyrosine residues which appear identical to the sites of phosphorylation catalyzed by pp60$^{v\text{-}src}$ in vitro [33].

The present work describes phosphorylation of two key regulatory enzymes, PFK and fructose 1,6-bisphosphatase, by the tyrosine kinase of the insulin receptor. Phosphorylation of these enzymes by viral oncogene products has not been reported. PFK and fructose 1,6-bisphosphatase differ from enolase, lactate dehydrogenase, and PGM in that they are also subject to serine phosphorylation in vivo and in vitro [24, 34–37]. The serine site of phosphorylation of both PFK and fructose-1,6-bisphosphatase is located near the carboxyl terminal of the enzymes [25,37,38]. Phosphorylation of PFK and fructose 1,6-bisphosphatase on these serine sites has been shown to modulate their enzyme activities either directly or indirectly (via modification of their affinity for allosteric effectors [39–43]. Thus, it is of interest to determine the location of the tyrosine site with reference to the serine site and to determine possible interactions of the two sites in the regulation of enzyme activity.

Regulation by both tyrosine and serine/threonine phosphorylation has been described for both the insulin receptor and the epidermal growth factor (EGF) receptor. Autophosphorylation on tyrosine residues activates the insulin receptor kinase while phosphorylation on serine/threonine induced by phorbol esters appears to be inactivating [44,45]. Similar opposing effects of tyrosine and serine/threonine phosphorylation have also been reported for the EGF receptor kinase [46].

It is not known whether PFK is phosphorylated on tyrosine residues in vivo. Earlier studies with intact cell systems have reported only serine phosphorylation of

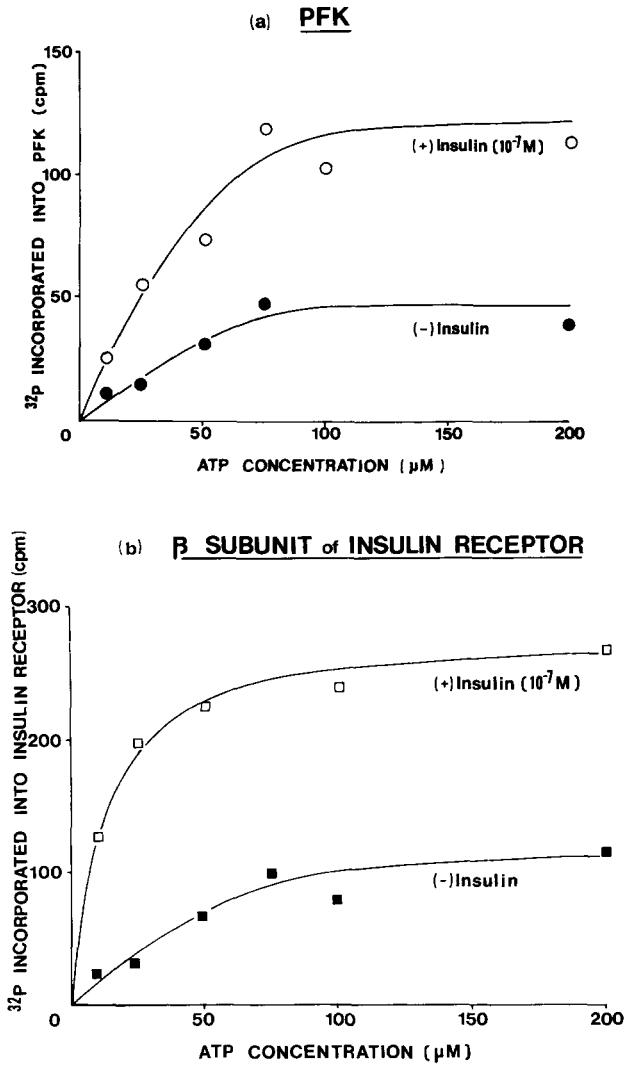

Fig. 7. Effect of ATP concentration on the phosphorylation of (a) phosphofructokinase and (b) β-subunit of the insulin receptor. Following preincubation of the insulin receptor with 5 mM $MnCl_2$ in the absence (−) or presence (+) of 10^{-7} M insulin, 4 μg purified PFK was added, and phosphorylation was initiated with [γ−^{32}P]ATP at the final concentrations indicated. After 15 min, the reaction was terminated and ^{32}P incorporated into the protein bands of 80KDa (PFK) and 95KDa (β-subunit of insulin receptor) separated by SDS gel electrophoresis was determined as described in Figure 3.

the enzyme [34,35]. In light of the results presented here, it would be of interest to determine whether tyrosine phosphorylation also occurs, in particular, in cells in response to insulin (or indeed, in cells transformed by $pp60^{v\text{-}src}$ or other retroviruses). This is possible since incubation of rat epididymal fat pads with insulin is known to result in an increase in the phosphorylation of PFK from 0.2 molP/enzyme tetramer to 0.4 molP/enzyme tetramer [47]. Furthermore, the apparent Km of PFK for tyrosine

phosphorylation of the enzyme by the insulin receptor in vitro is approximately 0.1 μM (Fig. 6), ie, within the range of concentrations of PFK in many cells. Phosphotyrosine could have been missed in previous studies since insulin-stimulated conditions were not studied. Thus far, however, in preliminary studies with antiphosphotyrosine antibodies, we have not detected any insulin-stimulated, tyrosine phosphorylation bands corresponding to the glycolytic/gluconeogenic enzymes in either hepatocytes or L6 muscle cells [48]. Recently, Kamps, Buss, and Sefton [49] have also cast doubt on the role of cytoplasmic protein tyrosine phosphorylation in the effects of tyrosine kinases by showing that a Rous sarcoma virus transforming protein which lacked myristic acid phosphorylated various polypeptide substrates in the cell but did not bind to membranes or induce transformation.

At present, the precise effects of tyrosine phosphorylation of glycolytic/gluconeogenic enzymes are unknown. Based on the known concentration of PFK and the measured specific activity of [γ-^{32}P]ATP, the stoichiometry of ^{32}P incorporated into tyrosine residues of PFK in vitro was approximately 0.01 molP/mol enzyme tetramer. This value is equivalent to that reported for phosphorylation of other proteins by other tyrosine kinases [50,51], but in contrast to all other glycolytic enzyme substrates studied so far PFK is of regulatory importance. This enzyme catalyzes a key rate-limiting step in glycolysis [26–28], and flux through this reaction is increased in response to insulin in the presence of viral transformation of cells [20,21]. Possible ways by which tyrosine phosphorylation may modulate PFK are as follows: (a) changing enzyme activity, ie, through regulation by allosteric effectors or through changes in the polymeric state of the enzyme; (b) affecting the enzyme turnover rate; or (c) altering the enzyme's location and/or its association with other proteins. Preliminary experiments in our laboratory have shown that incubation of purified PFK with insulin receptor purified to homogeneity by insulin-Sepharose affinity chromatography results in significant changes in the activity of PFK and may occur through modulation of the aggregation status of the enzyme [52]. This raises the possibility that tyrosine phosphorylation of this enzyme may alter its enzymatic properties.

ACKNOWLEDGMENTS

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REFERENCES

1. Kasuga M, Karlsson FA, and Kahn CR: Science 215:185–187, 1982.
2. Kasuga M, Zick Y, Blithe DL, Karlsson FA, Haring H-U, and Kahn CR: J Biol Chem 257:9891–9894, 1982.
3. Van Obberghen E, and Kowalski A: FEBS Lett 143:179–182, 1982.
4. Petruzzelli LM, Ganguly S, Smith CR, Cobb MH, Rubin CS, and Rosen OM: Proc Natl Acad Sci USA 79:6792–6796, 1982.
5. Avruch J, Nemenoff RA, Blackshear PJ, Pierce MW, and Osathanondh R: J Biol Chem 257:15162–15166, 1982.
6. Haring H-U, Kasuga M, and Kahn CR: Biochem Biophys Res Commun 108:1538–1545, 1982.

7. Kasuga M, Fujita-Yamaguchi Y, Blithe DL, and Kahn CR: Proc Natl Acad Sci USA 80:2137–2141, 1983.
8. Kasuga M, Fujita-Yamaguchi Y, Blithe DL, White MF, and Kahn CR: J Biol Chem 258:10973–10980, 1983.
9. Shia MA, Pilch PF: Biochemistry 22:717–721, 1983.
10. Van Obberghen E, Rossi B, Kowalski A, Gazzano H, and Ponzio G: Proc Natl Acad Sci USA 80:945–949, 1983.
11. Roth RA, Cassell DJ: Science 219:299–301, 1983.
12. Zick Y, Whittaker J, Roth J: J Biol Chem 258:3431–3434, 1983.
13. Rosen OM, Herrera R, Olowe Y, Petruzelli LM, Cobb MH: Proc Natl Acad Sci USA 80:3237–3240, 1983.
14. Heldin C-H, Westermark B: Cell 37:9–20, 1980.
15. Cooper JA, Hunter T: Mol Cell Biol 1:165–178, 1981.
16. Cooper JA, Hunter T: Mol Cell Biol. 1:394–407, 1981.
17. Eigenbrodt E, Fisher P, Rubsamen H, and Friis RR: EMBO J 2:1565–1570, 1983.
18. Cooper JA, Reiss NA, Schwartz RJ, Hunter T: Nature (London) 302:218–223, 1983.
19. Halperin ML, Denton RM: Biochem J 113:207–214, 1969.
20. Morell B, Froesch ER: Eur J Clin Invest 3:119–123, 1973.
21. White MF, Haring H-U, Kasuga M, Kahn CR: J Biol Chem 259:255–264, 1984.
22. Laemmli UR: Nature (London) 227:680–685, 1970.
23. Grigorescu F, White MF, Kahn CR: J Biol Chem 258:13708–13716, 1983.
24. Riquelme RT, Hosey MM, Marcus F, Kemp RG: Biochem Biophys Res Commun 85:1480–1487, 1978.
25. Kemp RG, Foe LC, Latshaw SP, Poorman RA, Henrikson RL: J Biol Chem 256:7282–7286, 1981.
26. Newsholme EA, Randle PJ: Biochem J 80:655–662, 1961.
27. Passoneau JV, Lowry DH: Biochem Biophys Res Commun 7:10–15, 1962.
28. Wu R: Biochem Biophys Res Commun 14:79–85, 1964.
29. Haring H-U, Kasuga M, White MF, Crettaz M, Kahn CR: Biochemistry 23:3298–3306, 1984.
30. Zick Y, Kasuga M, Kahn CR, Roth J: J Biol Chem 258:75–80, 1983.
31. Denton RM, Brownsey RW, Belsham GJ: Diabetologia 21:347–362, 1981.
32. Presk P, Glossman H, Eigenbrodt E, Schoner W, Rubsamen H, Friis R, Bauer H: Cancer Res 40:1733–1741, 1980.
33. Cooper JA, Esch FS, Taylor SS, Hunter T: J Biol Chem 259:7835–7841, 1984.
34. Riquelme RT, Fox RW, Kemp RG: Biochem Biophys Res Commun 81:864–870, 1978.
35. Kagimoto T, Uyeda K: J Biol Chem 254:5584–5587, 1979.
36. Riou JP, Claus TH, Flockhart D, Corbin JD, Pilkis SJ: Proc Natl Acad Sci USA 74:4619–4619, 1977.
37. Hosey M, Marcus F: Proc Natl Acad Sci USA 78:91–94, 1981.
38. Riquelme RT, Kemp RG: J Biol Chem 255:4367–4371, 1980.
39. Foe LG, Kemp RG: J Biol Chem 257:6368–6372, 1982.
40. Kitagima S, Sakakibara R, Uyeda K: J Biol Chem 258:13292–13298, 1983.
41. Ekdahl KN, Ekman P: FEBS Lett 167:203–209, 1984.
42. Meek DW, Nimmo HG: Biochem J 222:125–130, 1984.
43. Sale EM, Denton RM: Biochem J 232:897–904, 1985.
44. Rosen OM, Herrera R, Olowe J, Petruzzelli LM, Cobb MH: Proc Natl Acad Sci USA 80:3237–3240, 1983.
45. Takayama S, White MF, Lauris V, Kahn CR: Proc Natl Acad Sci USA 81:7797–7801, 1984.
46. Hunter T: Nature 311:414–416, 1984.
47. Sale EM, Denton RM: Biochem J 232:905–910, 1985.
48. Okamoto M, White MF, Kahn CR: Manuscript in preparation.
49. Kamps MP, Buss JE, Sefton BM: Cell 45:105–112, 1986.
50. Cooper JA, Hunter TJ: J Biol Chem 256:1108–1115, 1983.
51. Sefton BM, Hunter T, Ball EH, Singer SJ: Cell 24:165–174, 1981.
52. Sale EM, Fujita-Yamaguchi Y, Kahn CR: submitted for publication.