Glucose Stimulates Translocation of the Homeodomain Transcription Factor PDX1 from the Cytoplasm to the Nucleus in Pancreatic β-Cells*

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One of the mechanisms whereby glucose stimulates insulin gene transcription in pancreatic β-cells involves activation of the homeodomain transcription factor PDX1 (pancreatic/duodenal homeobox-1) via a stress-activated pathway involving stress-activated protein kinase 2 (SAPK2, also termed RK/p38, CSBP, and Mxi2). In the present study we show, by Western blotting and electrophoretic mobility shift assay, that in human islets of Langerhans incubated in low glucose (3 mM) PDX1 exists as an inactive 31-kDa protein localized exclusively in the cytoplasm. Transfer of the islets to high (16 mM) glucose results in rapid (within 10 min) conversion of PDX1 to an active 46-kDa form that was present predominantly in the nucleus. Activation of PDX1 appears to involve phosphorylation, as shown by incorporation of 32P into the 46-kDa form of the protein. These effects of glucose could be mimicked by chemical stress (sodium arsenite), or by overexpression of SAPK2 in the β-cell line MIN6. Overexpression of SAPK2 also stimulated PDX1-dependent transcription of a –50 to –250 region of the human insulin gene promoter linked to a firefly luciferase reporter gene. The effects of glucose were inhibited by the SAPK2 inhibitor SB 203580, and by wortmannin and LY 294002, which inhibit phosphatidylinositol 3-kinase (3-kinase, PI 3-kinase) activity, while stress (e.g. arsenite treatment) stimulates the SAPK2 pathway in β and other cell types by a mechanism independent of PI 3-kinase (16).

Recombinant PDX1, synthesized in Escherichia coli, has a molecular mass of 31 kDa, similar to that predicted from its amino acid sequence. It is inactive, insofar as it does not bind to its recognition sequence in the insulin promoter as measured by electrophoretic mobility shift assay. However, treatment of recombinant PDX1 with SAPK2 in the presence of Mg-ATP and a β-cell extract activates its DNA binding, concomitant with a shift in molecular mass from 31 kDa to 46 kDa (16). The molecular modifications responsible for this shift in molecular mass are as yet unclear. In the present study we show that PDX1 exists as the inactive 31-kDa form in the cytoplasm of human islets incubated in low (3 mM) glucose. Treatment with 16 mM glucose stimulates conversion to the active 46-kDa form, which is localized predominantly in the nucleus. These events are inhibited by SB 203580 (5 µM) (an inhibitor of SAPK2), wortmannin, and LY 294002 (inhibitors of PI 3-kinase), and mimicked by sodium arsenite and overexpression of SAPK2.

EXPERIMENTAL PROCEDURES

Chemicals and Reagents—Radiochemicals were purchased from Amersham International (Slough, Berks, United Kingdom (UK)), sodium arsenite from Fisons (Loughborough, UK), and wortmannin from Sigma (Poole, UK). SB 203580 was a generous gift from Dr. J. Lee and Dr. P. Young (SmithKline Beecham, King of Prussia, PA). Anti-PDX1 antibody was kindly provided by Dr. C. V. Wright (Vanderbilt University, Nashville, TN). Anti-SAPK2 antibody was purchased from New England Biolabs (Cambridge, UK). Anti-upstream stimulatory factor (USF) antibody was kindly provided by Dr. M. Sawadogo (University of Texas, Houston, TX).

Oligonucleotides—Oligodeoxynucleotides were purchased from Alta Bioscience (University of Birmingham, Birmingham, UK). Single-stranded complementary oligonucleotides were annealed as described

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†The abbreviations used are: PI 3-kinase, phosphatidylinositol 3-kinase; USF, upstream stimulatory factor; EMSA, electrophoretic mobility shift assay; PAGE, polyacrylamide gel electrophoresis; IL, interleukin; NF, nuclear factor.
Previously (18), and labeled with [γ-32P]ATP using T4 polynucleotide kinase. Oligonucleotide B, corresponding to the 3′ site of the human insulin gene promoter, is a complementary double-stranded 30-mer 5′-CCCTGCTGTTAAGACTTGAAGCCGCCTGG-3′.

Isolation and Treatment of Human Islets of Langerhans—Human islets of Langerhans were obtained from pancreata of human organ donors, and all procedures were carried out with the approval of the appropriate Ethical Committee. The pancreatic duct was cannulated in situ and digestion achieved by intraductal infusion of collagenase. The islets were then separated from contaminating acinar tissue by centrifugation at 7000 rpm for 30 s. The cell containing 0.5 mM glucose. Cells were then removed from the wells and a with a 5-h preincubation in Dulbecco's modified Eagle's medium con-

Cell Culture—MIN6 cells were grown in Dulbecco's modified Eagle's medium containing 5 mM glucose, supplemented with 15% heat-inacti-

Luciferase Assay—30 μl of cell extract was added to 350 μl of buffer A, pH 7.8 (15 mM MgSO4, 30 mM glycylglycine, 2 mM Na3ATP) containing 0.45 μM coenzyme A and 2.56 mM Triton X-100. To this, 150 μl of buffer G (30 mM glycylglycine) containing 0.5 mM luciferin (Sigma) was injected and the luminescence read at 560 nm using a Berthold Luminescence reader. Protein content of the cell extract was measured by taking 6 μl of extract and adding 144 μl of water and 150 μl of Coomassie protein assay reagent (Pierce). Protein content was read at a wavelength of 620 nm using a Titer Tech Multiscan MCC340. A standard curve of known bovine serum albumin protein concentrations was used to calculate the protein concentration of the samples.

Western Blotting—For Western blot analysis, 1-μg samples of cell extract were fractionated by SDS-PAGE, blotted onto to ECL-nitrocellu-

RESULTS

Initial experiments were undertaken to determine the effect of glucose on the molecular mass and intracellular location of PDX1. Human islets of Langerhans were incubated for 5 h in 3 mM glucose, transferred to 16 mM glucose, and PDX1 analyzed at various time intervals by Western blotting of whole cell extracts. In 3 mM glucose PDX1 was present as a 31-kDa protein (Fig. 1A). Within 10 min in 16 mM glucose, the 31-kDa protein was converted to the 46-kDa form. Conversion was complete within 15 min. To investigate the effect of glucose on the intracellular location of the two forms of PDX1, human islets were incubated in 3 or 20 mM glucose and cytoplasmic and nuclear fractions prepared. In 3 mM glucose, the 31-kDa form was present in the cytoplasm; however, in 16 mM glucose, the 46-kDa form was present in the cytoplasm and the nucleus (Fig. 1B). Treatment with acid phosphatase converted the 46-koenzyme A and 2.56 mM Triton X-100. To this, 150 μl of buffer G (30 mM glycylglycine) containing 0.5 mM luciferin (Sigma) was injected and the luminescence read at 560 nm using a Berthold Luminescence reader. Protein content of the cell extract was measured by taking 6 μl of extract and adding 144 μl of water and 150 μl of Coomassie protein assay reagent (Pierce). Protein content was read at a wavelength of 620 nm using a Titer Tech Multiscan MCC340. A standard curve of known bovine serum albumin protein concentrations was used to calculate the protein concentration of the samples.

Western Blotting—For Western blot analysis, 1-μg samples of cell extract were fractionated by SDS-PAGE, blotted onto to ECL-nitrocellulose membrane (Amer sham), and incubated for 60 min in a buffer containing 10 mM Tris-HCl, 0.05% (v/v) Tween 20, 0.5 mM NaCl, and a 1:5000 dilution of anti-PDX1 antibody. The antigen-antibody complex was then detected by incubating the membrane for another 60 min in a buffer containing a 1:5000 dilution of horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody (ECL, Amer sham).
PDX1 is rapidly modified by high glucose concentrations in human islets of Langerhans. A, Western blot analysis of human islets of Langerhans incubated in 3 mM glucose for 5 h, then transferred to 20 mM glucose for the time periods indicated. Whole cell extracts were prepared, and probed with a specific anti-PDX1 antibody. B, Western blot analysis of cytoplasmic (C) and nuclear (N) extracts prepared from human islets of Langerhans incubated in 3 mM glucose for 5 h, or 3 mM glucose for 5 h followed by 20 mM glucose for 30 min. In lane 5, high glucose nuclear extract was incubated for 30 min with 10 units of potato acid phosphatase (AP). C, electrophoretic mobility shift assay of cytoplasmic (C) and nuclear (N) samples prepared from human islets of Langerhans incubated in 3 mM glucose for 5 h, or 3 mM glucose for 5 h followed by 20 mM glucose for 30 min, using the A3 site of the human insulin gene promoter as a probe.

As shown previously (16), glucose stimulates the transcriptional activity of a DNA construct containing the firefly luciferase gene under the control of the thymidine kinase reporter, which is driven by a human insulin gene fragment from −60 to −260 (pGL-LUC200) (Fig. 5). Overexpression of SAPK2 in MIN6 cells had no effect on the control vector pGL-LUC, which lacked the insulin gene fragment, but gave a 5-fold stimulation of pGL-LUC200 activity, similar to that seen with 16 mM glucose (Fig. 5). The effect of overexpression of SAPK2 on the LUC200 activity was inhibited by SB 203580 (10 μM), confirming that these effects were the direct consequence of the higher SAPK2 activity in the transfected cells.

DISCUSSION

We have previously shown that glucose activates PDX1 DNA binding activity and insulin promoter activity in pancreatic β-cells via a pathway involving SAPK2 (RK/p38). We also showed that SAPK2 in vitro activated recombinant PDX1 DNA binding activity, concomitant with a change in the molecular mass of PDX1 from 31 kDa to 46 kDa (16). In the present study, we show that glucose stimulates the conversion of PDX1 from a 31-kDa unphosphorylated to a 46-kDa phosphorylated form in isolated human islets of Langerhans (Fig. 1) and in MIN6 cells (Fig. 4). The activation of PDX1, and translocation to the nucleus, were inhibited by SB 203580 and stimulated by arsenite and overexpression of SAPK2. This strongly supports a role for SAPK2 in these events. That glucose stimulates a rapid translocation of PDX1 to the nucleus is compatible with the results of a recent study showing that glucose stimulates translocation of a c-Myc-tagged PDX1 (IPF-1) from the nuclear periphery to the nucleoplasm (22).
resent the critical step in glucose-induced translocation to the nucleus and activation of DNA binding activity. However, it is unclear whether phosphorylation alone accounts for the observed change in size. It is possible that phosphorylation allows or promotes a second event, which is yet to be characterized. Phosphorylation may induce a conformational change in PDX1 that affects its mobility in SDS-PAGE, but the change in size could equally result from a further post-translational modification. Many transcription factors contain nuclear localization signals within their amino acid sequences, but others, such as PDX1, do not contain recognizable nuclear localization signals and may require modification at the post-translational level.
Common modifications promoting nuclear localization include glycosylation (23), and ubiquitination (24, 25), which modifies proteins through the addition of ubiquitin-like protein species such as SUMO1 (small ubiquitin-related modifier 1). In the case of RanGAP (Ran-GTPase activating protein) (25), phosphorylation allows the addition of SUMO1, which alters the apparent molecular mass of the protein by 20 kDa, and promotes its translocation from the cytoplasm to the nucleus. Similar events may be occurring with PDX1.

Increased binding activity of transcription factors through phosphorylation can occur by several mechanisms. Many transcription factors become phosphorylated in the cytoplasm, stimulating movement into the nucleus (26). However, this is not always the case. For example, insulin stimulates the nuclear translocation of several kinases, such as mitogen-activated protein kinase (27), which can then directly phosphorylate nuclear protein substrates, or activate other kinases (28). In the present study SAPK2 is shown to be present exclusively in the cytoplasm, suggesting that the events governing PDX1 activation occur exclusively in the cytoplasm, promoting translocation of an active PDX1 into the nucleus.

Glucose activation of PDX1 nuclear translocation occurs through a β-cell signaling pathway involving PI 3-kinase, and resulting in the activation of SAPK2. Hence, overexpression of SAPK2 can artificially promote PDX1 translocation to the nucleus even in lower glucose concentrations. In fact, overexpression of SAPK2 stimulated PDX1 binding activity to levels higher than those seen in high glucose (Fig. 4). This hyperstimulation of PDX1 by overexpression of SAPK2 occurred in 0.5 or 20 mM glucose, and appeared to reflect activation of PDX1 at maximum efficiency. In untransfected β-cells endogenous SAPK2 may represent a rate-limiting step in PDX1 activation, with glucose eliciting a controlled stimulation. Overexpression of SAPK2 to artificially high levels may therefore result in a hyperstimulation of the PDX1 activation pathway, resulting in an increased ability to phosphorylate and translocate all available PDX1 protein. This “hyperstimulation” of PDX1 binding activity did not appear to be reflected in the levels of insulin gene transcription relative to those observed in high glucose. This is not surprising, as PDX1 alone does not control transcription of the insulin gene. Several other factors (e.g. E1 and E2 site binding factors) are absolutely required for transcription of the insulin gene. Increased PDX1 binding activity will only exert an effect in the presence of these factors, and a maximum response would require their combined activities.

Glucose stimulates PDX1 phosphorylation and nuclear translocation through SAPK2. PDX1 is not unique in this respect; CREB, which also binds to the human insulin gene promoter (15, 16), has its binding activity controlled through alteration in its phosphorylation status. Recent studies in SK-N-MC cells have shown that CREB-dependent transcription is triggered by co-transfection with SAPK2 (29), which may act in this case through stimulation of a downstream kinase such as MAPKAPK2 (30). However, SAPK2 has been shown to directly phosphorylate several transcription factors, including ELK1, CHOP, and MEF2C (17). Stress-activated protein kinases have also been implicated in the nuclear localization of other transcription factors. In EL-4 D676 cells, IL-1 activation of interleukin-1 receptor-associated kinase and of stress-activated pro-
The presence of 20 mM glucose concentrations on pGL-Luc200 reporter gene activity.

MIN6 cells were transfected with the control construct pGL-Luc (lanes 1–4), or with pGL-Luc200 (lanes 5–8), and were incubated in 0.5 mM (white) or 16 mM glucose (black), or 0.5 mM glucose in the presence of 20 mM SB 203580 (hatched). In lanes 3, 4, and 7–9, cells were co-transfected with SAPK2 as indicated.

Fig. 5. Overexpression of SAPK2 mimics the effect of high glucose concentrations on pGL-Luc200 reporter gene activity. A, Western blot analysis of cytoplasmic (C) and nuclear (N) extracts prepared from MIN6 cells overexpressing SAPK2, using a specific anti-SAPK2 antibody. SAPK2 has an apparent molecular mass of 38 kDa. B, MIN6 cells were transfected with the control construct pGL-Luc (LUC, lanes 1–4), or with pGL-Luc200 (LUC200, lanes 5–8), and were incubated in 0.5 mM (white) or 16 mM glucose (black), or 0.5 mM glucose in the presence of 20 mM SB 203580 (hatched). In lanes 3, 4, and 7–9, cells were co-transfected with SAPK2 as indicated.

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