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Rapid report

Insulin induces PFKFB3 gene expression in HT29 human colon adenocarcinoma cells

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Abstract

Fructose 2,6-bisphosphate is present at high concentrations in many established lines of transformed cells. It plays a key role in the maintenance of a high glycolytic rate by coupling hormonal and growth factor signals with metabolic demand. The concentration of fructose 2,6-bisphosphate is controlled by the activity of the homodimeric bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK-2). We report here the PFKFB-3 gene expression control by insulin in the human colon adenocarcinoma HT29 cell line. The incubation of these cells with 1 μM insulin resulted in an increase in the PFK-2 mRNA level after 6 h of treatment, this effect being blocked by actinomycin D. Furthermore, insulin induced ubiquitous PFK-2 protein levels, that were evident after a lag of 3 h and could be inhibited by incubation with cycloheximide.

Keywords: Glycolysis; Fructose 2,6-bisphosphate; 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase; Gene expression

The metabolism of many tumors and neoplastic or transformed cell lines is characterized by their high rate of glycolysis even under aerobic conditions [1]. The specific regulatory mechanisms responsible for enhanced glycolysis and the novo nucleic acid synthesis in transformed cells have remained largely obscure. A stimulation of glycolysis can also be observed following exposure of quiescent cells to growth and tumorigenic factors [2,3]. The discovery of fructose 2,6-bisphosphate (Fru-2,6-P_2), a potent activator of 6-phosphofructo-1-kinase (PFK-1) [4–7], has focused attention on this important control point of the glycolytic pathway. Indeed, Fru-2,6-P_2 is present at high concentrations in many neoplastic cells where it plays a key role in the maintenance of a high glycolytic rate [2,4–7].

The concentration of Fru-2,6-P_2 is controlled by the homodimeric bifunctional enzyme 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase (PFK-2) that is responsible for both the formation and degradation of this compound [4–7]. Since the discovery of rat liver PFK-2, several mammalian isozymes have been identified in different tissues. These isozymes are generated by alternative splicing from only four genes, designated PFKFB 1–4 [7–10].

Stimulation of glycolysis was observed in chick embryos [11] and human fibroblasts [12,13] and in HT29 colon adenocarcinoma cells [14,15] upon exposure to mitogenic concentrations of insulin. Stimulation of lactate production by insulin was found to

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correlate with Fru-2,6-P_2 level and PFK-2 activity [11,15]. The mechanism of PFK-2 activation by insulin was not known, although a stimulation of transcription and/or translation had been suggested [2]. HT29 cells, derived from a human colon adenocarcinoma, display a very active glycolytic pathway and insulin produces an induction of Fru-2,6-P_2 concentration, PFK-2 activity and lactate production [15]. We have now examined if these changes induced by insulin involve the transcriptional regulation of the ubiquitous PFKFB-3 gene. In fact, PFKFB-3 codes for a PFK-2 isozyme present in proliferating and transformed cells that displays a high kinase/bisphosphatase activity ratio that may be essential for tumor cell growth [16–19].

Exponentially growing HT29 cells were maintained during 24 h in serum-free medium to induce their quiescence and then stimulated with 1 μM insulin or phosphate buffered saline (PBS). As shown in Fig. 1A, a specific band corresponding to the product of PFKFB-3 gene (ubiquitous PFK-2 mRNA) was detected in HT29 cells by Northern blot hybridization using as a probe a PFKFB-3-specific 3′ cDNA region [17]. The incubation of HT29 cells with 1 μM insulin resulted in a 2.2 ± 0.2-fold increase in PFK-2 mRNA levels after 6 h of treatment. Incubation of HT29 cells with an inhibitor of RNA transcription (actinomycin D) blocked the insulin dependent PFK-2 mRNA expression, showing that this effect was at the transcriptional level. Cycloheximide, an inhibitor of protein synthesis, had a minor inhibitory effect on PFK-2 mRNA expression (Fig. 1B), suggesting the possible participation of some new translated protein in this insulin effect.

Recently, Chesney et al. cloned an inducible isoform of PFK-2 that only differs from the ubiquitous isoform in a 23-nucleotide insertion in the 3′ end of the coding region of the mRNA [19]. These two PFK-2 cDNA products are generated through alternative splicing of the same gene (PFKFB-3) [20]. The 23-nucleotide differentially spliced exon of the inducible isoform causes a change of the reading frame giving rise to a protein that differs in the C-terminal amino acid sequence and it is inducible by lipopolysaccharide (LPS) in monocytes [19]. In order to determine if HT29 cells expressed inducible PFK-2, we attempted a RT-PCR amplification of total RNA obtained from these cells. The RT-PCR only amplified a single band of the same length as the positive control, corresponding to the ubiquitous cDNA (Fig. 1C). Cycle sequencing of the PCR amplified products showed that only the ubiquitous isoform was present in these cells (data not shown).

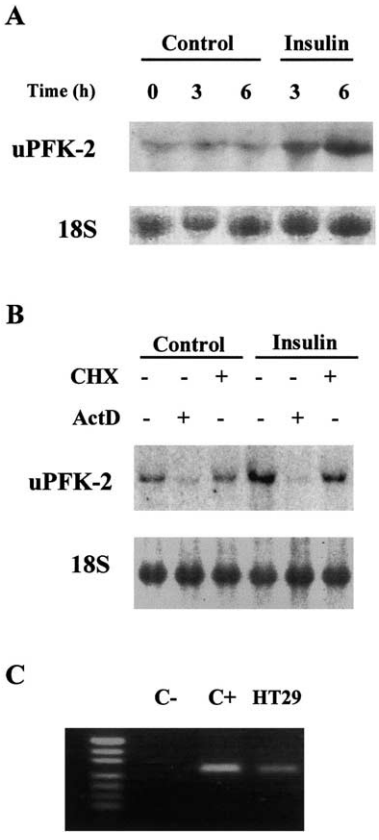

Fig. 1. Effect of insulin on PFKFB-3 mRNA expression. (A) HT29 cells were incubated with insulin (1 μM) for 3 or 6 h. (B) HT29 cells treated for 6 h with insulin and 35 μM cycloheximide (CHX) or 2.5 μg/ml actinomycin D (ActD). Total RNA was extracted and analyzed by Northern blot with a specific probe for ubiquitous PFK-2 [17]. (C) RT-PCR amplification. Two micrograms of total RNA from HT29 cells were reverse-transcribed using a Ready-To-Go T-Primed First-Strand Kit (Amersham Pharmacia Biotech) and oligo(dT) as primer. PCR amplification was carried out using 2 μl of the reverse-transcribed RNA product from HT29 cells (HT29 lane) or uPFK-2 cDNA (C+ lane) and the gene-specific oligonucleotides LR-5 (5′-CACACACCGGGAGAGG-3′, corresponding to position 1434 bp of ubiquitous PFK-2 cDNA) and LR-3 (5′-TCGTTCCCATGAGGCGTC-3′, corresponding to position 2191 bp), in a GeneAmp PCR System 2400 from Perkin-Elmer. All panels are representative of three different experiments.

To evaluate the modulation exerted by insulin on ubiquitous PFK-2 protein levels, we generated a specific antibody against ubiquitous PFK-2 by subcuta-

neous immunization of white New Zealand rabbits with an ubiquitous PFK-2 carboxy-terminal peptide ((NH$_2$)-CMKGSRSSADSSRKH-(COOH)) that was conjugated to keyhole limpet hemocyanin. This peptide comprises a unique region of the ubiquitous PFK-2 (amino acids 507–520) that differs significantly from the corresponding portion of inducible PFK-2 [19]. This antibody does not recognize the liver or testis isoform overexpressed in COS-1 cells (Fig. 2A) that were identified by an antibody against liver PFK-2. As shown in Fig. 2B, HT29 cell incubation in the presence of insulin produced an increase in ubiquitous PFK-2 protein concentration that was evident after a lag of 3 h (Fig. 2C). This induction was inhibited by incubation of HT29 cells with cycloheximide, showing that translation was necessary (Fig. 2D).

It has been reported that insulin stimulates glucose uptake and lactate production in HT29 cells [14,15]. The accumulation of Fru-2,6-P$_2$ which is induced by insulin in these cells is concomitant with the increase of PFK-2 activity and glycolysis [15], and this effect closely resembles that observed in other transformed cell lines [11–13,19,21,22]. The results herein reported demonstrate that the PFK-2 isozyme present in HT29 cells is the ubiquitous PFK-2 isoform coded by the PFKFB-3 gene. Moreover, they also show that insulin controls PFK-2 in these cells mainly by the transcriptional regulation of PFKFB-3 gene, since addition of actinomycin D completely blocked its induction. These results also explain those previously presented that demonstrated that PFK-2 from HT29 cells was different from the liver isozyme and shared some common characteristics with the heart isoform [15].

Insulin is a major regulator of cell glucose metabolism and controls the expression of specific genes. Despite the importance of insulin in gene regulation, little is known about the *cis*-elements and *trans*-acting factors which mediate insulin action [23]. Recently, it has been shown that the transcription factor sterol regulatory binding protein-1c (SREBP-1c) is transcriptionally stimulated by insulin in the liver and can mediate insulin action on the expression of glucokinase and lipogenesis-related genes through binding to sterol regulatory elements (SRE, 5′-TCACCCCCAC-3′) and E-boxes (5′-CANNTG-3′) [24–26]. Several SRE sequences and E-boxes, identified in the promoter region of the PFKFB 3 gene [20], could be responsible for this action. Direct involvement of these elements on the regulation by insulin must be further evaluated.

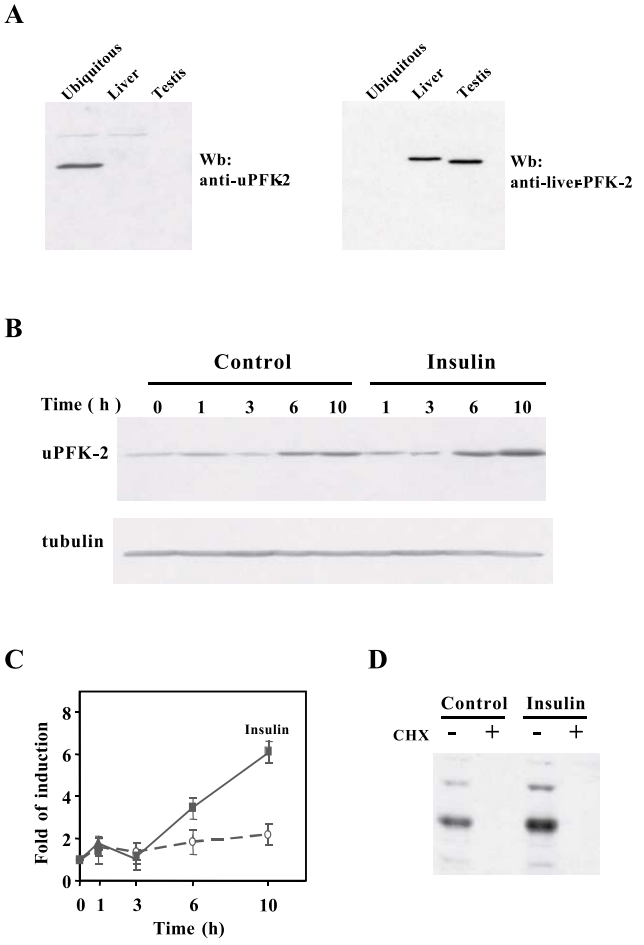

Fig. 2. Effect of insulin on PFK-2 protein levels. (A) Western blot of COS cell extracts overexpressing PFK-2 cDNA of ubiquitous, liver or testis isozyme, using an anti-ubiquitous PFK-2-specific antibody (left) or anti-liver PFK-2 one [27] (right). (B) HT29 cells were treated with 1 μM of insulin at different times. (C) The densitometric data of B are represented. Each point represents the mean ± S.E.M. of the data obtained by densitometric scanning from three independent experiments using monoclonal antibody anti-β-tubulin (from Oncogene) for normalization. (D) HT29 cells were treated for 6 h with insulin in the presence or absence of 35 μM cycloheximide. Protein lysates were analyzed by Western blot.

In conclusion, the results now reported show that ubiquitous PFK-2 isozyme is present in HT29 cells and that its gene is transcriptionally regulated by insulin, playing a key role in the maintenance of high glycolytic rates in this cell line derived from a human colon adenocarcinoma.

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