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Transcriptional regulation of rat liver protein disulphide-isomerase gene by insulin and in diabetes

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The mRNA encoding for rat protein disulphide-isomerase (PDI) increases 3-fold in the liver of diabetic rats and is accompanied by similar changes at the protein level. Long treatment (for 3 days) of diabetic rats with insulin reverses this effect of diabetes both at the mRNA and protein levels. The higher expression of rat PDI mRNA in diabetes is due to an increase in the transcriptional rate of the gene, and insulin treatment of diabetic animals produces within 30 min a decrease in the level of transcription of PDI gene, as judged by nuclear run-on transcription experiments performed *in vivo*. These results clearly show a role for insulin in the regulation of transcription of the gene encoding this multifunctional protein in rat liver.

INTRODUCTION

Protein disulphide-isomerase (PDI) is an endoplasmic-reticulum enzyme (EC 5.3.4.1), also known as thiol:protein disulphide-isomerase and glutathione:insulin transhydrogenase, that seems to participate in correct disulphide-bond formation in secreted proteins (Anfinsen & Scheraga, 1975; Freedman *et al.*, 1984; Bulleid & Freedman, 1988). Recent studies have shown, by comparison among cDNA-derived amino acid sequences, that PDI (Edman *et al.* 1985), the β-subunit of prolyl 4-hydroxylase (Pihlajaniemi *et al.*, 1987), a tri-iodothyronine-binding protein of the endoplasmic reticulum (Cheng *et al.*, 1987; Yamauchi *et al.*, 1987), a thyroxine 5'-monodesiodase (Boado *et al.*, 1988) and the glycosyltransferase presenting protein (Gaetha-Habib *et al.*, 1988) are products of the same gene, indicating that it is a multifunctional protein with several catalytic functions. From now on we will refer to this multicatalytic protein simply as 'PDI'.

We were interested in the regulation of gene expression by insulin, and we followed two approaches to clone new mRNAs the expression of which was under insulin control: (1) differential screening of a liver cDNA library with cDNA probes derived from normal and diabetic poly(A)$^+$ liver mRNA (Mira & Castaño, 1989) and (2) direct cloning of rat liver glucokinase (GK), an enzyme known to be under insulin control (Weinhouse, 1976). The first step in cloning GK cDNA was the purification of the rat liver enzyme by published procedures (Holroyde *et al.*, 1976). The GK preparation obtained was not homogeneous and contained, on the basis of SDS/PAGE, a major 57 kDa band that we thought could correspond to GK, as this value was within the reported molecular mass (50–60 kDa) for this enzyme (Weinhouse, 1976). This protein band was cut out from a preparative SDS/PAGE gel and used to obtain polyclonal antibodies by injection into rabbits. We obtained an antibody that failed to immunoprecipitate GK activity, but recognized the 57 kDa protein by immunoblot; later we found that this protein was increased during diabetes, whereas GK is known to be decreased (Weinhouse, 1976). Further work with the antibody, molecular cloning and sequencing of the cDNA encoding this 57 kDa protein show that the protein recognized by the antibody was protein disulphide isomerase. The increased amount of PDI in the diabetic liver prompted us to study the regulation of the expression of PDI by insulin and in diabetes. To our knowledge this is the first report of the transcriptional regulation by hormones of the gene encoding this multifunctional protein.

METHODS

Materials

Enzymes for nucleic acid modification were from Boehringer or New England Biolabs. Radiolabelled compounds were from Amersham International. Oligonucleotide primers for sequencing and random primers were from Pharmacia. Insulin was from Novo. All other reagents were of analytical-reagent grade or molecular-biology grade.

Animals and treatments

Male Wistar rats (150–200 g body wt) were used, fed on stock diet and water *ad libitum*, and housed under controlled conditions providing light from 7:00 to 19:00 h. Diabetes was induced by intravenous injection of streptozotocin (Sigma) (60 mg/kg body wt.) in 20 mM-sodium acetate buffer (pH 4.5)/0.15 M-NaCl. Diabetes was initially checked by glucosuria, and only rats with blood glucose levels over 300 mg/dl were used in all the experiments, 3 or 10 days after streptozotocin injection.

Purification of rat liver PDI and production of a polyclonal antibody

We obtained PDI by using the protocol for the purification of rat liver GK described by Holroyde *et al.* (1976), obtaining, in the final step of purification, a GK specific activity of 20 units/mg of protein (below the reported 120 units/mg) and showing, by SDS/PAGE, several bands and a major one (40%) of 57 kDa that we thought could correspond to rat liver GK. This protein band was extracted from preparative SDS/PAGE gels and injected into rabbits. A polyclonal antibody was obtained that recognized, by immunoblot, this 57 kDa band, but failed to immunoprecipitate GK (M_r 52000) activity from a 100000 *g* rat liver supernatant. Further characterization of the antibody presented in the Results section shows us that the protein we

Abbreviations used: PDI, protein disulphide-isomerase; α_2m, α_2-microglobulin; GK, glucokinase; poly(A)$^+$, polyadenylated; FCS, fetal-calf serum.
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injected and to which we obtained a polyclonal antibody was actually PDI and not GK.

Protein immunoblotting

Liver samples were homogenized (1:10, w/v) in 50 mM-Tris/HCl (pH 7.5)/0.15 M-NaCl/1% (v/v) Triton X-100 and centrifuged at 100000 *g* for 60 min at 4 °C; under these conditions all PDI protein is solubilized (results not shown). Protein was measured in the supernatant by the Bradford (1976) procedure.

Proteins were analysed by SDS/10%-(w/v)-PAGE as described by Laemmli (1970) or by gel electrophoresis as described by O'Farrell (1977) and transferred to nitrocellulose (BA85; Schleicher and Schuell) as described by Towbin *et al.* (1979). Nitrocellulose filters were blocked overnight with blocking buffer [50 mM-Tris/HCl (pH 7.5)/0.5 M-NaCl/3% (w/v) BSA], incubated with anti-(rat PDI) antibody at 1:200 dilution for 3 h at room temperature with shaking, washed three times (10 min each) with blocking buffer without BSA, and then the second antibody, a peroxidase-coupled goat anti-rabbit antibody (Copper) at 1:200 dilution, was added. Incubation was continued for 2 h at room temperature with shaking, and, after three washes (10 min each) with blocking buffer without BSA, the blot was developed with 4-chloro-1-naphthol as a dye. Quantification was done by densitometric scanning of the negatives of the corresponding immunoblots.

Cell immunofluorescence

NRK cells were grown on coverslips in Dulbecco's Modified Eagle's medium supplemented with 10% (v/v) fetal-calf serum (FCS). After the cells had grown to 50% confluency, the coverslips containing the cells were fixed and permeabilized with 100% methanol at −20 °C for 15 min, rinsed three times with saline (0.9% NaCl) and used for indirect immunofluorescence. First antibody was added at 1:200 dilution in saline containing 10% FCS and incubated with the cells for 3 h at room temperature; after three washes (10 min each) with saline, the second antibody, a rhodamine-conjugated goat anti-rabbit antibody (Copper), was added at 1:200 dilution in saline containing 10% FCS. Incubation with the second antibody was for 2 h at room temperature; the coverslips were then washed with saline three times (10 min each) and mounted in saline containing 50% (v/v) glycerol and examined under a Nikon fluorescence microscope. Controls with preimmune rabbit serum or the second antibody alone were negative.

Immunological screening of a rat liver expression library

A λgt11 rat liver cDNA library, kindly provided by Dr. Frank Gonzalez [National Cancer Institute, National Institutes of Health (NCI, NIH), Bethesda, MD, U.S.A.], was used for immunoscreening with the rabbit polyclonal antibody against rat liver PDI (at 1:100 dilution) preabsorbed with Y1090 *Escherichia coli* extract. As second antibody, a peroxidase-labelled goat anti-rabbit antibody (Copper) was used. Approx. 500000 phages in 20 plates of 140 mm were transferred to nitrocellulose circles. After four rounds of purification, three positive individual phages were isolated; the DNA of these phages was prepared, digested with restriction endonuclease *Eco*R1 and their inserts subcloned into the *Eco*R1 site of plasmid pUC 18. DNA sequencing of the cDNAs was done by the dideoxy method of Sanger *et al.* (1977) and the method of Maxam & Gilbert (1980).

Experiments *in vivo*

Diabetic rats (3 or 10 days after streptozotocin injection) were injected subcutaneously with lente insulin (Novo); 6 units/200 g body wt, twice a day with a 12 h interval, for 3 days (or saline to the controls). Glucosuria was tested daily, and only the animals that showed normal glycaemia were used for experiments. At the end of the treatment, the animals were killed by decapitation and a liver biopsy was taken (1–2 g), immediately frozen in liquid N_2 and stored at −70 °C until used for RNA or protein extraction.

In the study of the time course of the effect of insulin on PDI mRNA levels, two different animals for each time point were injected intravenously at zero time with 5 units of insulin Actrapid MC (Novo)/200 g body wt. and subcutaneously with 5 units of lente insulin (Novo). Liver biopsies were taken at 0, 2 and 4 h and used for RNA extraction.

RNA extraction

RNA was extracted by the guanidium chloride (BRL) method described by Adams *et al.* (1977).

^{32}P-labelled probes

The corresponding plasmid was digested with the appropriate restriction enzyme and the insert was purified by low-melting-point-agarose [Bethesda Research Laboratories (BRL)] gel electrophoresis (Maniatis *et al.*, 1982). Approx. 100–200 ng of insert DNA was used for labelling with [α-^{32}P]dCTP by the random-priming method (Feinberg & Vogelstein, 1984). Probes were purified from unincorporated label by the spin-column method as described by Maniatis *et al.* (1982). Routinely the specific radioactivities of the probes were $(5–10) \times 10^8$ c.p.m./μg of DNA.

Probes used were a 1.2 kb *Eco*R1 fragment of plasmid p1914 of PDI cDNA corresponding to the 5′ end of the mRNA or a 1.1 kb *Eco*R1 fragment of plasmid p1912 corresponding to the 3′ end of the mRNA (see the Results section), a 0.6 kb *Eco*R1 fragment of plasmid p4104 corresponding to the cDNA of α_2-microglobulin (α_2m) (Mira & Castaño, 1989), and a 0.6 kb *Eco*R1–*Hin*dIII fragment of rat β-actin cDNA kindly provided by Dr. Bruce Patterson (NCI, NIH).

Northern- and slot-blot analysis

Total RNA was electrophoresed on 1.4%-agarose/2.2 M-formaldehyde gels in the presence of 20 mM-Mops/5 mM-sodium acetate/1 mM-EDTA, pH 7.0, and transferred to NYTRAN membranes (Schleicher und Schuell), as recommended by the manufacturer. Slot blots were prepared by filtering the appropriate amounts of total RNA on NYTRAN membranes in a mini-fold slot-blot apparatus (BRL). In both cases the filters were baked at 80 °C for 2 h, prehybridized for 4 h at 68 °C in 4 × SSC (1 × SSC is 15 mM-sodium citrate/0.15 M-NaCl, pH 7.0), 10 × Denhardt's (100 × Denhardt's contains, in 100 ml, 2 g of Ficoll-400, 2 g of polyvinylpyrrolidone and 2 g of BSA), 0.1% SDS and 200 μg of denatured salmon sperm DNA/ml and hybridized for 12 h at 68 °C with the appropriate probe. The filters were washed twice for 15 min with 2 × SSC/0.1% SDS and twice for 15 min with 0.1 × SSC/0.1% SDS at 68 °C. The filters were air-dried and exposed to X-ray film with an intensifying screen at −70 °C for 12–48 h. The same filters were washed according to manufacturer's recommendations to remove one probe and re-used to hybridize with another probe under the same conditions, except in the results presented in Fig. 3, where the first probe (PDI) was washed but not removed before adding the second probe (α_2m), and the autoradiogram presented is unique in showing both mRNAs detected in the same blot and with the same time of exposure. Quantification was done by scanning densitometry of the X-ray film, and the results presented are the averages from at least three different experiments, taking the hybridization with the β-actin probe as a control.

Transcription *in vitro* in isolated nuclei and hybridization of nuclear labelled RNAs to plasmid DNA

Isolation of liver nuclei from normal animals, diabetic animals, diabetic animals treated by intravenous injection of insulin (10 units/200 g body wt.), run-on transcription reactions and isolation of labelled nuclear RNA were performed as described by Lamers *et al.* (1982).

Plasmid DNAs (2 μg) were digested with *Eco*R1, denatured with 0.5 M-NaOH for 10 min at room temperature and bound to nylon filters (NYTRAN; previously soaked in 1.5 M-NaCl/0.5 M-NaOH) with a dot-blot apparatus. The filters were air-dried, washed with 5 × SSC for 15 min at room temperature and baked for 2 h at 80 °C. Prehybridization was done at 42 °C in a final volume of 0.4 ml containing 5 × SSPE (1 × SSPE is 0.15 M-NaCl/10 mM-sodium phosphate/1 mM-EDTA, pH 7.4), 50% (v/v) formamide, 5 × Denhardt's, 0.5 mM-UTP and 200 μg of denatured salmon sperm DNA/ml. After 2 h the filters were hybridized for 36 h at 42 °C in 0.4 ml of hybridization solution (5 × SSPE/50% formamide/5 × Denhardt's) with identical amounts of labelled RNAs obtained from the run-on transcription from the different conditions under assay [(1–3) × 10^7 c.p.m.] as judged by trichloroacetic acid precipitation of the isolated labelled RNAs. After hybridization the filters were washed twice (15 min each) with 5 × SSC/2% SDS/0.5 mM-EDTA at 42 °C, once with 2 × SSC, once with 2 × SSC containing 40 μg of RNAase A/ml for 30 min at 25 °C and once with 1 × SSC for 20 min and subjected to autoradiography. Quantification of the results was done by densitometric scanning of the autoradiograms; the changes reported were calculated as a quotient of the values obtained for PDI or α_2m divided by the values obtained for β-actin used as control.

RESULTS

Molecular cloning of rat liver PDI

We obtained a rabbit polyclonal antibody against a 57 kDa protein present in our GK preparation after four chromatographic steps [see Holroyde *et al.* (1976) for the purification of

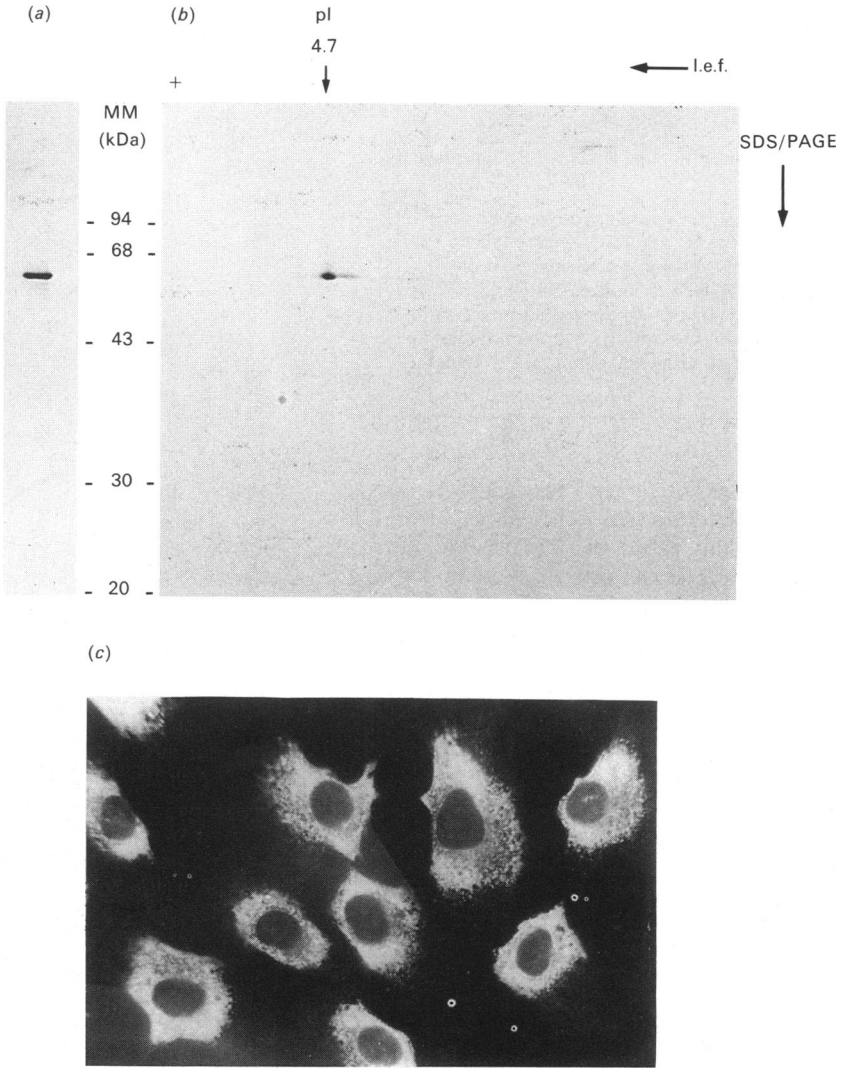

Fig. 1. Characterization of the polyclonal antibody against rat liver PDI

(*a*) and (*b*) show immunoblots of total proteins (20 μg) solubilized from rat liver and separated by one- or two-dimensional gel electrophoresis as described in the Methods section. pI markers used for isoelectric focusing (I.e.f.) in the first dimension (*b*) were from USB, and molecular-mass (MM) markers were from Bio-Rad. (*c*) Photomicrograph of the immunofluorescence of NRK cells with the polyclonal antibody shown in (*a*) and (*b*). Indirect immunofluorescence was achieved as described in the Methods section. The antiserum was used at 1:200 dilution. Preimmune serum from the same rabbit and second antibody alone gave no fluorescence. Magnification × 1000.

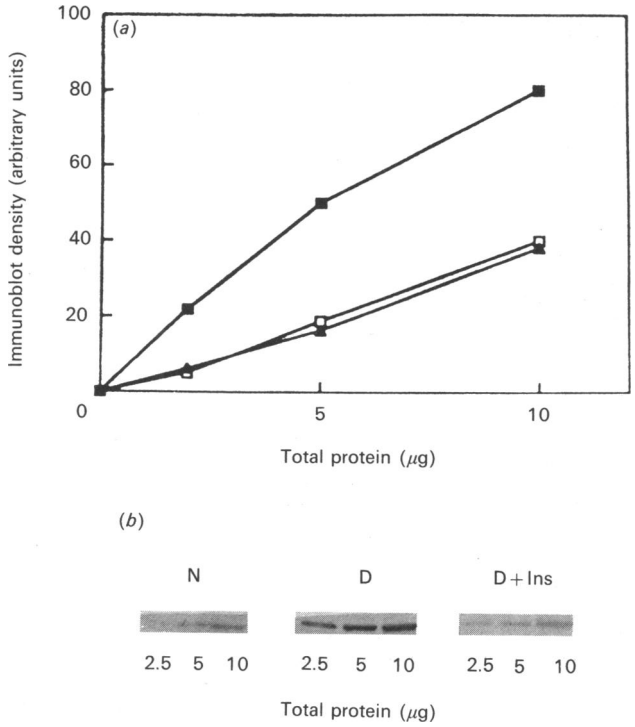

Fig. 2. Effect of diabetes and insulin on the levels of PDI protein by immunoblot analysis

Different amounts of total solubilized protein from liver of normal rats [N (□)], 10-day-diabetic rats [D (■)] or 10-day-diabetic rats treated with insulin for 3 days [D+Ins (▲)] were separated on 10% (w/v) polyacrylamide gels, transferred to nitrocellulose and probed with the anti-(rat PDI) antibody. (*b*) Shows the relevant part of the immunoblot. (*a*) Shows the quantification of immunoblots by densitometric scanning. Further experimental details are given in the Methods section.

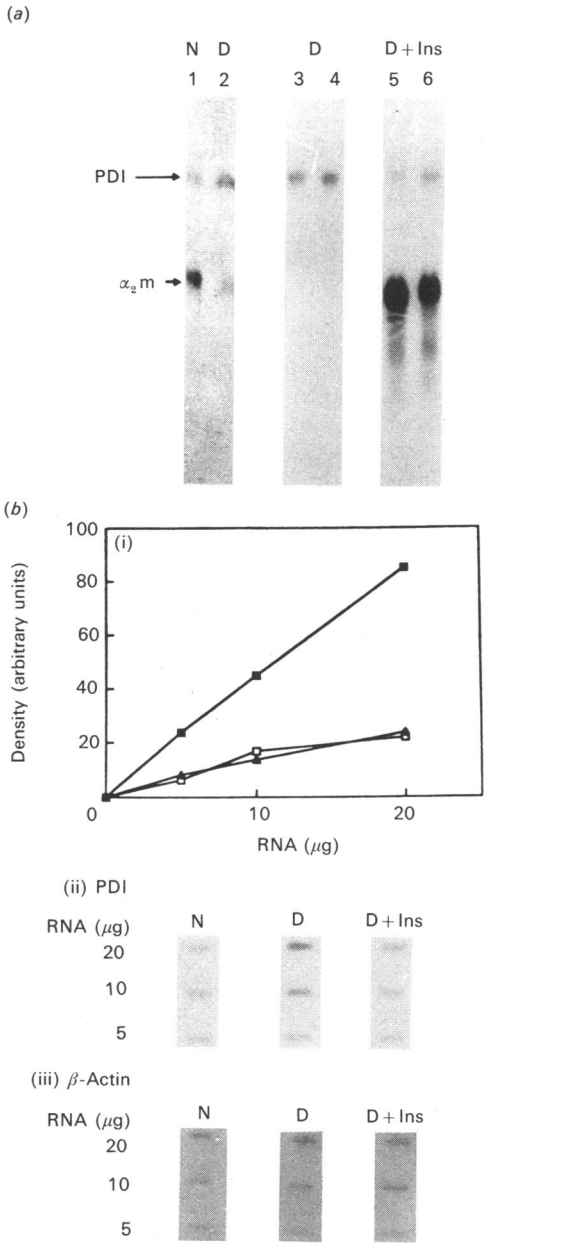

Fig. 3. Northern- and slot-blot analysis of the effect of diabetes and insulin treatment on PDI mRNA levels and α_2m

(*a*) Total RNA (25 μg) from liver of normal rats (N, lane 1), rats diabetic for 3 days (D; lane 2) or for 10 days (D; lanes 3 and 4; two different animals) or 10-day-diabetic rats treated with insulin for 3 days (D+Ins; lanes 5 and 6; two different animals) was analysed by Northern blot and hybridized first with rat PDI cDNA and afterwards with α_2m cDNA probes and exposed for autoradiography (see the Methods section). The size of the mRNAs shown correspond to the correctly sized messages [2.8 kb for PDI mRNA (Edman *et al.*, 1985) and 1.3 kb for α_2m mRNA (Unterman *et al.*, 1981). (*b*) Different amounts of total liver RNA, as indicated in the Figure, from normal rat (N), diabetic rats (10 days) (D) or diabetic rats (10 days) treated with insulin for 3 days (D+Ins) were analysed by slot blot and hybridized with PDI cDNA (ii) and with β-actin (iii) as a control. (i) Shows the quantification of the slot-blot hybridization. The results are quotients between the values obtained for PDI hybridization and those obtained for β-actin as a control. For further experimental details, see the Methods section.

GK] that we thought corresponded to GK. The antibody we obtained failed to immunoprecipitate the GK activity from a liver soluble extract, but this result did not surprise us, because we used a denatured protein as antigen. The antibody recognized by immunoblot a protein of 57 kDa (Fig. 1*a*) with a pI of 4.7 (Fig. 1*b*), and by indirect immunofluorescence this protein was localized in the endoplasmic reticulum (Fig. 1*c*). These results, together with the high abundance of the 57 kDa protein in the microsomal fraction of the liver, the presence of this protein in many rat liver tissues and in NRK cells that lack GK activity, and the increase of this protein in liver in diabetes (see below), are in contrast with GK, which is a soluble enzyme, is present only in rat liver and pancreas (Iynedjian *et al.*, 1986) and decreases during diabetes (Weinhouse, 1976). All these data clearly indicate to us that we have obtained a polyclonal antibody against a 57 kDa microsomal protein that is not GK.

The polyclonal antibody was used for immunoscreening of a λgt11 rat liver cDNA library. We purified three phages; full sequencing of their inserts and comparison against the EMBL/GenBank/DDBJ Nucleotide Sequence Databases showed identity with the reported rat liver PDI sequence (Edman *et al.*, 1985). Two of the phages contained a unique *Eco*R1 fragment that went from the internal *Eco*R1 site to the 3′ end of the reported PDI sequence, and one of them contained two *Eco*R1 fragments: one identical with the above-mentioned fragment and the other going from the 5′ end of the mRNA to the internal *Eco*R1 site (Edman *et al.*, 1985). The only significant discrepancy found with the published nucleotide sequence was residue 20 of the coding sequence (shown below), where proline is found in the published sequence (Edman *et al.*, 1985) and we found two codons, in agreement with published sequence of the

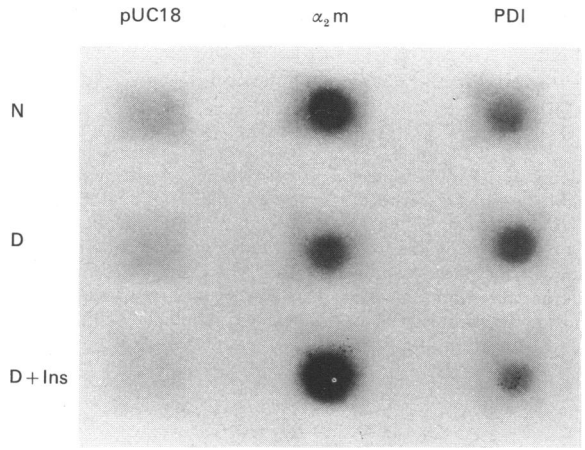

Fig. 4. Effect of diabetes and insulin treatment on the nuclear transcription *in vitro* of the PDI gene

Nuclei were isolated from normal rats (N), diabetic (3 days) (D) or diabetic rats (3 days) treated with insulin (10 units)/200 g body wt. for 30 min (D+Ins). *In vitro* run-on transcription experiments and hybridization to a filter containing plasmids were done as described in the Methods section. The Figure shows the autoradiogram after hybridization with labelled RNA transcripts of filters containing no insert (pUC18), p1914 cDNA of PDI (PDI) or p4104 α_2m cDNA (α_2m).

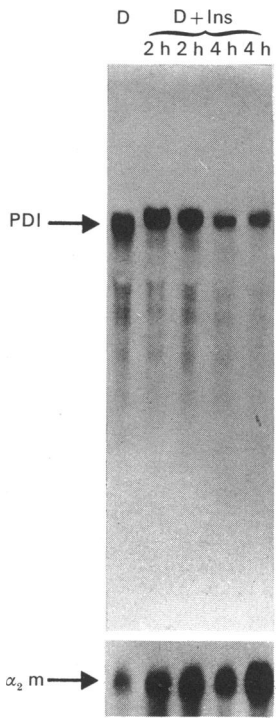

Fig. 5. Time course of the effect of insulin treatment of diabetic rats on the levels of PDI mRNA

Rats diabetic for 3 days were either not treated (D) or treated with insulin (D+Ins) for 2 or 4 h (two different animals each time point) as described in the Methods section. Total RNA was isolated, and 20 μg was analysed by Northern blot and hybridized with PDI or α_2m cDNA.

human β-subunit of prolyl 4-hydroxylase (Pihlajaniemi *et al.*, 1987):

	20	21	
	Glu	Ala	Leu	Ala
	Glu		***Pro***	Ala
*GAG	**GCG**	**CTG**	GCG
†GAG		***CCG***	GCG
‡GAG	**GCG**	**CTG**	GCG

* Present report.
† Rat PDI sequence (Edman *et al.*, 1985).
‡ β-Subunit of human prolyl 4-hydroxylase (Pihlajaniemi *et al.*, 1987).

The antibody was able to recognize the fusion proteins encoded by the phage cDNAs and the cDNA was able to select a mRNA whose '*in vitro*' translated product was immunoprecipitable by the previously characterized antibody (results not shown), confirming that the antibody obtained was against rat liver PDI.

Effect of diabetes and insulin treatment on PDI protein levels

An increase in the amount of PDI in the liver of 10-day diabetic rats was observed when compared with those in normal rats and returned to normal levels after prolonged (3 days) treatment with insulin (Fig. 2). The same effect was observed in animals kept diabetic for 3 days and treated with insulin for 3 days (results not shown). The values obtained by quantification of the immunoblots in three different experiments, expressed in arbitrary densitometric units, were between 10 and 15 (normal), 40 and 50 (diabetic) and 12 and 16 (diabetic, treated with insulin) for 5 μg of total liver protein, so there was a 3–5-fold increase in the PDI protein levels in diabetes that was reversed by treatment with insulin.

Effect of diabetes and insulin treatment on rat liver PDI mRNA levels

The observed effect of diabetes on the amount of PDI protein is also reflected at the mRNA level, as shown in Fig. 3. Fig. 3(*a*) shows that both long (3 days) as well as prolonged (10 days) diabetes produced an increase in the level of PDI mRNA in liver. Treatment with insulin (3 days) of diabetic animals restored this mRNA to normal levels. In the same blot, and for comparison, hybridization with α_2m is presented; α_2m mRNA behaves in the opposite manner, decreasing (10 times) in diabetes and increasing in response to insulin (Mira & Castaño, 1989; Roy *et al.*, 1980). Quantification of this experiment by slot-blot analysis is presented in Fig. 3(*b*). The values obtained by quantification of the slot blots in three different experiments, expressed in arbitrary densitometric units, were between 12 and 17 (normal), 40 and 50 (diabetic) and 14 and 16 (diabetic, treated with insulin) for 10 μg of total RNA, so there was a 3–4-fold increase in the level of PDI mRNA during diabetes that was reversed after insulin treatment.

This 3–4-fold increase in diabetes is unlikely to be due to an increase in the level of glucose or to increased levels of glucagon or decreased levels of tri-iodothyronine in the diabetic animal, since no changes in the level of mRNA for PDI was detected in animals fed with 10% (w/v) glucose in the drinking water for 4 days, in animals starved for 72 h or in hypothyroid rats (results not shown). This last result is in agreement with that in a recent report showing no effect of tri-iodothyronine on PDI mRNA in GH3 cells (Obata *et al.*, 1988).

Effect of diabetes on the run-on nuclear transcription of the PDI gene *in vitro*

The observed increase in the level of PDI mRNA in diabetic rats could be explained by an increase at the level of transcription of this gene or by stabilization of PDI mRNA in diabetes. To clarify this point we performed run-on transcription experiments

in vitro with nuclei isolated from normal animals, diabetic animals and diabetic animals treated with insulin (10 units/200 g body wt.) for 30 min. The labelled RNAs obtained were hybridized with filters containing different plasmids. The rate of transcription was assessed by autoradiography of the plasmid filters hybridized with the same amounts of total labelled RNAs. As Fig. 4 shows, there was an increase in the rate of transcription of the PDI gene during diabetes (about 3–4-fold), and the expected decrease (10-fold) in α_2m transcription (Mira & Castaño, 1989) was observed with the same labelled RNAs. Insulin treatment for 30 min decreased the level of transcription of the PDI gene to that observed in normal liver and, in parallel, there is an increase in the transcription of α_2m (Fig. 4).

Time course of insulin effect on the PDI mRNA levels in diabetic animals

As noted above, we observed a rapid decrease in the rate of transcription of the PDI gene after insulin treatment of diabetic animals; for that reason we decided to find out whether the same short-term effect of insulin could also be observed at the steady-state level of PDI mRNA in liver. We took diabetic animals and treated them with insulin as described in the Methods section and, at different times, liver biopsies were taken and total RNA isolated and analysed by Northern blot. Fig. 5 shows that, 2 h after insulin treatment, there was no change in the level of PDI mRNA, although there was a full recovery of the levels of α_2m mRNA. A 50% decrease in the level of PDI mRNA was observed 4 h after insulin treatment. These results indicate that the degradation of the PDI mRNA accumulated in the diabetic liver is not as rapidly responsive to the insulin treatment as was the decrease observed in the transcriptional rate of the PDI gene.

DISCUSSION

The aim of the present study was centred on the regulation of protein and mRNA levels of rat liver PDI during diabetes. The increased amounts of PDI protein during diabetes can be seen as a direct consequence of the increased levels of PDI mRNA in diabetic liver. Prolonged treatment of diabetic rats with insulin was required to reduce the amount of PDI protein to the levels found in normal rats; this could be explained by the long half-life of the protein in the endoplasmic reticulum (Ohba *et al.*, 1981) and, as a consequence, long treatment with insulin is required to reduce the amounts of mRNA accumulated during diabetes. The physiological significance of the increase of PDI protein during diabetes will require a detailed study of the changes of the enzymic activities reported to be associated with this protein [for a critical review, see Freedman (1989)]. Nevertheless, we should point out that the observed increase in PDI content during diabetes better correlates with collagen production, likely to increase during diabetes (Brownlee & Cerami, 1981), than with secreted proteins (Peavy *et al.*, 1978; Roy *et al.*, 1980; Jefferson *et al.*, 1983) and glycosylation (Sharma *et al.*, 1987), which are known to be decreased in diabetes.

The increased level of PDI mRNA in diabetes is clearly due to an increase of the rate of transcription of the gene, and insulin treatment of diabetic rats produces a fast decrease (within 30 min) in the rate of transcription of the PDI gene. This negative effect of insulin is similar to insulin inhibition of phosphoenolpyruvate carboxykinase (Cimbala *et al.*, 1982; Granner *et al.*, 1983) and growth-hormone (Prager & Melmed, 1988) gene transcription. The 5′ flanking region of the PDI gene may be useful for the identification of possible regulatory sequences involved in the negative regulation of transcription by insulin. Further experiments are required to test whether these sequences may be directly involved in the negative regulation of gene transcription by insulin.

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