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ARTICLE

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Glucose-induced changes of multiple mouse islet proteins analysed by two-dimensional gel electrophoresis and mass spectrometry

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Abstract *Aims/hypothesis:* The aim of this study was to investigate molecular mechanisms of glucose-induced changes in islets of Langerhans by analysing global changes in protein patterns of islets exposed to elevated glucose concentrations. *Methods:* Islets were isolated from C57BL/6J mice and used either directly or after exposure to 11 mmol/l glucose for 24 h. Islet protein profiles were obtained by two-dimensional gel electrophoresis, and protein spots were identified by peptide mass fingerprinting using mass spectrometry. *Results:* Two-dimensional gels of freshly isolated islets and islets exposed to 11 mmol/l glucose contained 1,074 and 1,254 spots, respectively. The number of differentially expressed spots was 379, with 20 spots appearing as new proteins in islets exposed to 11 mmol/l glucose. We identified 124 spots corresponding to 77 protein entries and generated a reference map from freshly isolated islets. Actin, alpha enolase, cytokeratin 8, endoplasmin, glucose-regulated proteins, heat shock proteins, peroxiredoxins, prohormone convertase 2, protein disulphide isomerase, superoxide dismutase, tubulin, and V-type H^+-ATPase (V1 subunit A) were upregulated in islets exposed to 11 mmol/l glucose. In contrast, exocrine proteins and secretagogin were downregulated in these islets compared with in freshly isolated islets. *Conclusions/interpretation:* The islet proteome approach revealed simultaneous changes in protein patterns of islets exposed to elevated glucose concentrations, indicating enhanced insulin synthesis, granular mobilisation and maturation, and increased stress response. The changes may be of relevance for the understanding of altered islet function in the hyperglycaemic state. It is expected that the islet reference map will become an important tool for dissecting multifactorial islet processes.

Keywords Cytokeratin · Glucose · Heat shock protein · Insulin biosynthesis · Islet · Proteomic · Secretory granule · Stress response · Two-dimensional gel electrophoresis

Abbreviations ACT: Actin · AMY2: Alpha-amylase · ATP6V1A: ATPase, H^+ transporting, lysosomal 70 M_r, V1 subunit A, isoform 1 · CEL: Bile-salt-activated lipase precursor · CPA1: Carboxypeptidase A1 precursor · CPB1: Carboxypeptidase B1 · CHAPS: 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulphonate · CTRA1: Chymotrypsin A · CTRB1: Chymotrypsinogen B precursor · 2-DGE: Two-dimensional gel electrophoresis · DTT: Dithiothreitol · ELA1: Elastase 1 · ELA2: Elastase 2 · ELA3B: Elastase IIIB precursor · ENO1: Alpha-enolase · GRP: Glucose-regulated protein · GSIS: Glucose-stimulated insulin secretion · HSC70: Heat shock cognate 71 M_r protein · HSP: Heat shock protein · IEF: Iso-electric focusing · IPG: Immobilised pH gradient · KRT8: Keratin, type II cytoskeletal 8 protein · MALDI: Matrix-assisted laser desorption/ionisation · MS: Mass spectrometry · NCBI: National Centre for Biotechnology Information · NL: Non-linear · NP-7: Tergitol · PCSK2: Prohormone convertase 2 · PDI: Protein disulphide isomerase · PEBP: Phosphatidylethanolamine-binding protein · PMF: Peptide mass fingerprinting · PNLIP: Triacylglycerol lipase · PNLIPRP1: Pancreatic-lipase-related protein 1 · PRDX: Peroxiredoxin · SCGN: Secretagogin · SDS: Sodium dodecyl sulphate · SOD1: Superoxide dismutase · TOF: Time-of-flight · TRA1: Endoplasmin · TUBA: Tubulin alpha · TUBB5: Tubulin beta 5

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Introduction

The ability of the islet of Langerhans to respond with increased insulin release when the ambient glucose concentration is elevated is of fundamental importance for glucose homeostasis [1]. In type 2 diabetes mellitus this ability is impaired, especially with reduced first-phase insulin secretion but also with reduced second-phase insulin secretion [2], which leads to postprandial hyperglycaemia. A main strategy to alleviate the disease has therefore been to mimic the initial glucose-stimulated insulin secretion (GSIS) by exogenous administration of the hormone [3]. Although such measures improve the glycaemic control, the mechanisms behind the deranged GSIS are still to a large extent unknown. In this context the observation that islets from *ob/ob* and KKA^y mice, which are animal models of type 2 diabetes mellitus [4, 5], and from C57BL/6J control mice demonstrate improved GSIS after exposure to elevated glucose concentrations [6–8] is of interest. Indeed, such beneficial effects on GSIS have been correlated to changes in expression of individual islet proteins such as glucose transporter 2, glucokinase and uncoupling protein 2 [9–11]. However, GSIS is a multifactorial event, which calls for approaches capable of determining multiple proteins simultaneously for the elucidation of molecular mechanisms responsible for changes in GSIS. In the present study we have used two-dimensional gel electrophoresis (2-DGE) and mass spectrometry (MS) to characterise changes in global islet protein expressions related to exposing islets to high glucose. This proteomic approach was used to explore mechanisms involved in enhanced GSIS observed after exposure of islets isolated from C57BL/6J mice to elevated glucose [8]. Global protein patterns obtained from such islets were compared with those of freshly isolated C57BL/6J mouse islets. Identities of differentially expressed proteins, which were obtained by peptide mass fingerprinting (PMF) and compiled into a protein reference map, show orchestrated changes of multiple mouse islet proteins.

Materials and methods

Chemicals and reagents Reagents of the highest purity commercially available and deionised water were used. Immobilised pH gradient (IPG) Ready Strips (11 cm), pH range 3–10 non-linear (NL) were purchased from Bio-Rad (Hercules, CA, USA). Acetonitrile, silver nitrate, sodium hydroxide, trifluoroacetic acid, thiourea, Triton X-100, ammonium hydroxide, citric acid monohydrate, glacial acetic acid, methanol, formaldehyde (37%) and tris were from Merck (Darmstadt, Germany). Urea and Pharmalyte 3 to 10 were from Amersham Biosciences (Uppsala, Sweden). Collagenase and HEPES were purchased from Boehringer Mannheim (Mannheim, Germany) and fetal calf serum was supplied by Gibco (Paisley, UK). All other chemicals were from Sigma (St. Louis, MO, USA).

Isolation and culture of islets Male C57BL/6J mice (3–5 months) were placed in a sealed container into which a stream of CO_2 was delivered. When the animals became unconscious, they were killed by decapitation. The peritoneal cavity was opened and the pancreas was excised and cut into small pieces, which were digested with collagenase to obtain free islets of Langerhans. The digestion buffer contained 3 mmol/l glucose. The procedures involving animals were in accordance with national and international laws for the care and use of laboratory animals and were approved by the local animal ethics committee. Isolated islets were either analysed directly or kept in culture for 1 or 7 days in RPMI 1640 medium containing 11 mmol/l glucose and supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin and 30 μg/ml gentamycin. The freshly isolated and cultured islets were washed twice with glucose-free phosphate buffer (pH 7.4) supplemented with protease inhibitor cocktail and were finally snap-frozen in liquid nitrogen and stored at −85°C until analysis.

Sample preparation The frozen 200 pooled islets from two to three mice (analytical gels) or 600–800 pooled islets from four to five mice (preparative gels) were resuspended in 100 μl of 1% Triton X-100 and 2% SDS and broken by water bath sonication. The samples were incubated at 4°C for 30 min and treated with a PlusOne 2-D Clean-Up Kit (Amersham Biosciences). The protein pellet was re-suspended in rehydration solution for the iso-electric focusing (IEF) and protein concentration was determined using a 2-D Quant Kit (Amersham Biosciences). The approximate protein amounts for the analytical and preparative gels were 100 and 500 μg, respectively.

Two-dimensional gel electrophoresis Individual 11-cm IPG strips, pH 3–10 NL, were rehydrated in 200 μl of sample solubilised in modified buffer containing 7 mol/l urea, 2 mol/l thiourea, 0.5% Triton X-100, 4% CHAPS, 0.5% pharmalyte (pH 3–10), 0.1% NP-7, protease inhibitor cocktail and 60 mmol/l DTT. Solubilisation was aided by sonication and incubation for 45 min at room temperature with constant mixing. The samples were then centrifuged and the supernatant was loaded onto the IPG strips. In-gel sample rehydration was allowed to proceed at 20°C for 15 h. The rehydrated strips were focused on the Protean IEF Cell (Bio-Rad) for about 35 kV·h at a maximum of 8.0 kV in rapid voltage ramping mode with a maximum current per strip of 50 μA. Equilibration and transfer of the IPG strips to the second dimension were done as described previously [12]. The SDS-PAGE was performed on 8 to 16% precast polyacrylamide gels (Criterion Gel System; BioRad). The gels were run at room temperature with a constant voltage of 120 V for 10 min, followed by 200 V for 60 min.

Protein visualisation The 2-D analytical gels were first stained with SYPRO Ruby (Molecular Probes, Eugene, OR, USA) and then with a silver staining method, which has been developed in our laboratory. The silver staining

protocol consists of the following: (1) fixation of gels in 50% methanol and 10% acetic acid (1 h); (2) washing in 5% methanol and 7% acetic acid (30 min); (3) washing in water (2×10 min); (4) staining with 100 ml of ammonical silver solution (0.075% sodium hydroxide, 0.35 N ammonium hydroxide and 0.8% w/v silver nitrate; 20 min); and (5) washing in water (3×5 min). The gel was developed in 100 ml of freshly prepared solution containing 0.005% citric acid and 0.019% formaldehyde until the desired intensity of staining was reached. The development was stopped by adding 10% acetic acid directly onto the developer (2–3 min). The gel was washed in water (5 min) and stored. This method is very sensitive (detection limit of 0.1 ng) and compatible with matrix-assisted laser desorption/ionisation (MALDI) time-of-flight (TOF) MS. The 2-D preparative gels were first stained with SYPRO Ruby and then with Bio-Safe Coomassie stain according to the manufacturer's instructions (Bio-Rad).

Image analysis The stained gels were imaged using a GS-800-calibrated densitometer (Bio-Rad). Raw scans were processed by the 2-D gel analysis software, PDQuest (Bio-Rad). For between-gel comparisons, a set of spot generation conditions (faint spot, small spot and largest spot cluster) was used. Spot patterns of different gels were matched to each other and each spot was given a unique identification number. The quantity of each spot was normalised to the total density of all matched spots within the gel to minimise the effect of experimental factors on protein spots. To find spots that differed quantitatively between freshly isolated and cultured islets, average intensities of resolved spots were compared using quantitative, qualitative and statistical functions within the PDQuest software. For reliable matching, 33 different landmark proteins were manually added in each gel. All the quantitatively different spots were also verified manually. Histogram information provided by the PDQuest software was generated for all valid spots remaining after removal of streaks, speckles and artefacts and was used to validate spot detection and differential expression analysis errors. Significant changes between spots were determined using Student's *t*-test for non-paired observations. Changes with a p value of less than 0.05 were considered statistically significant.

Determination of molecular weight and pI Molecular weight and pI for the individual proteins on the gels were interpolated from 2-D SDS-PAGE Standards (Bio-Rad), which consisted of multiple proteins with a molecular weight of between 17.5 and 76 M_r and a p*I* range of 4.5–8.5. A 2-DE gel that contained 5 µl of the diluted 2-D SDS-PAGE Standards was analysed in parallel to determine the position of the standard proteins in the islet proteome gels. When the molecular weight and p*I* values of each standard protein were provided, the PDQuest software automatically calculated the experimental molecular weight and p*I* of each protein spot.

Protein identification by mass spectrometry Mass spectrometry and protein identification were carried out by the Wallenberg Consortium North Expression Proteomics Facility (Department of Medical Biochemistry and Microbiology, Uppsala University, Sweden). Protein spots were excised from gels and in-gel digestion was performed with trypsin essentially as described previously [13]. Peptides were cleaned using ZipTip microcolumn (C18; Millipore, Bedford, MA, USA) following the instructions provided by the manufacturer. Samples for MALDI-MS analysis were prepared using α-cyano-4-hydroxy-trans-cinnamic acid as matrix. Mass spectra were recorded on a Bruker Reflex IV MALDI-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany). All mass spectra were internally calibrated with trypsin autolysis products; amino acid sequence 108–115 (MH^+=842.51) and sequence 58–77 (MH^+=2,211.10) and masses of known contaminants, e.g. keratin, were removed. Proteins were identified by PMF with the search program Mascot (Matrix Science, London, UK). The National Centre for Biotechnology Information (NCBI) number was used as the protein sequence database and the peptide masses were compared with the theoretical peptide masses of all available proteins from the species *Mus musculus*. The criteria used to accept identifications included the extent of sequence coverage, number of peptides matched, molecular weight search (MOWSE) score and whether the theoretical molecular weight and p*I* of the matched protein were within the experimental molecular weight±20% and experimental p*I* value±1.00

Protein information Information about identified proteins and putative functions was found at the ExPASy Molecular Biology Server at SWISS-PROT and at the NCBI, which were accessed between November 2002 and August 2003.

Results

Global protein patterns of freshly isolated mouse islets and islets exposed to elevated glucose Proteins were extracted from freshly isolated islets and islets exposed to 11 mmol/l glucose for 24 h and separated by 2-DGE. Such analytical gels, which were obtained from approximately 200 islets, were stained with silver (Fig. 1). While 1,074 spots were observed in gels of freshly isolated islets, 1,254 spots were detected in the islets exposed to 11 mmol/l glucose for 24 h. The mean densities of the spots were obtained by averaging the densities of the corresponding spots of gels of freshly isolated islets (n=6) and islets exposed to elevated glucose concentration for 24 h (n=6), respectively. The spot density measurements were also performed in SYPRO-stained gels (data not shown). By using both staining procedures, advantage was taken of the high sensitivity of the silver staining and the wide linear range of the SYPRO Ruby staining [14]. Also, spots that showed negative staining or the doughnut phenomenon in silver-stained gels could be reliably determined in SYPRO-Ruby-stained gels. Indeed, when gel replicates of the freshly isolated islets or islets exposed to elevated glucose concentration for 24 h were matched by PDQuest, a cor-

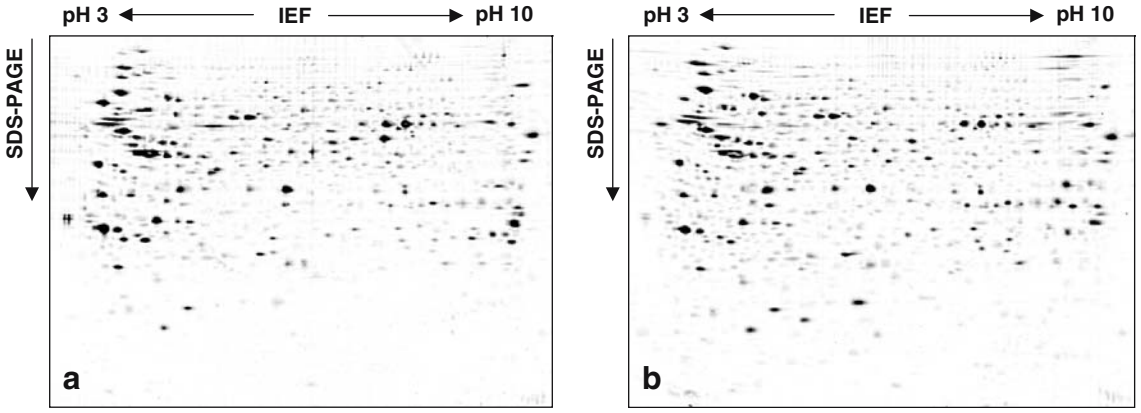

Fig. 1 Representative 2-D gels of six separate experiments of freshly isolated (**a**) and cultured (**b**) islets. Approximately 100 μg of islet proteins were separated on an 11-cm IPG strip (pH 3–10 NL), followed by 8–16% SDS-PAGE. Proteins were detected by silver staining, and image analysis of scanned gels was carried out using the PDQuest software

relation coefficient of 0.75 or more was obtained. Analysis between freshly isolated islets and islets exposed to 11 mmol/l glucose for 24 h revealed that 379 spots were two-fold or more differentially expressed. While 187 spots were upregulated, 192 spots were downregulated in islets exposed to 11 mmol/l glucose for 24 h compared with in freshly isolated islets. Further qualitative analysis of the 379 spots revealed that six of these spots were barely visible in gels of islets exposed to 11 mmol/l glucose for 24 h but had densities at least ten-fold greater than the minimum number of detectable spots in gels of the freshly isolated islets. Also, 20 other spots among these 379 spots were barely visible in gels of freshly isolated islets but had densities at least ten-fold greater than the minimum number of detectable spots in the islets exposed to 11 mmol/l for 24 h.

Protein identification and construction of a reference map Identities of the mapped proteins were obtained from preparative gels, which were stained with SYPRO Ruby yielding around 560 spots. Subsequently, the gels were stained with colloidal Coomassie. In total, 182 spots were manually excised from gels of both freshly isolated islets (n=2) and islets exposed to 11 mmol/l glucose for 24 h (n=2), and 124 spots, which corresponded to 77 distinct proteins, were characterised by PMF. In the remaining 58 spots, no significant identification was obtained. The excised spots were selected among the differentially expressed proteins but also among other proteins. The latter spots were selected to ensure range in pI and molecular weight, of which some coincided with already identified proteins demonstrating concordance of the present map with previously published maps [15–17]. A representative MALDI-TOF PMF spectrum of the heat shock protein 60 (HSP60) is presented in Fig. 2. Our gel spot identification yield was 70%, with several proteins appearing at multiple positions on the gels. Overall, values of the spots based on gel-estimated molecular weight and pI matched well with the corresponding theoretical values. The identified proteins were entered under their gene names into a single protein map (Fig. 3). This 2-D gel image is representative of the highly reproducible and resolvable islet protein pattern obtained from freshly isolated mouse islets and has been used as a reference map. In this protein map, all spots from the preparative gels are combined except the spots that are present only in the cultured islets. The pI values of the identified proteins varied between 4 and 9 except for two proteins, which had pI values of between 9 and 10. The molecular weight of the identified proteins varied between 10 and 100 M_r with the exception of one protein with larger mass than 100 M_r. The identified proteins including those obtained from the gels of islets exposed to 11 mmol/l glucose for 24 h are available electronically (ESM). The identified proteins were categorised with regard to both function and location. The major functional groups were proteins involved in metabolism (25%), signalling (20%), cellular defence and molecular chaperones (18%), exocrine enzymes (15%) and structural proteins (7%). With regard to location, 44% were cytosolic, 17% mitochondrial and 11% endoplasmic reticular. However, many of the identified proteins have multiple functions and subcellular locations.

Islet protein expression changes after exposure to elevated glucose concentration The expression levels of the spots representing the identified 77 proteins were determined in the analytical gels of freshly isolated islets and islets exposed to 11 mmol/l glucose for 24 h. These expression levels and their fold changes, which were calculated by dividing the average spot quantities recorded in islets exposed to 11 mmol/l glucose for 24 h with quantities recorded in freshly isolated islets, are summarised in Table 1. Proteins with at least a two-fold change in expression level, when islets exposed to 11 mmol/l glucose for 24 h were compared with freshly isolated islets, included 58 M_r glucose-regulated protein (GRP58), actin (ACT), alpha enolase (ENO1), endoplasmin (TRA1), heat shock protein cognate 74 (HSP74), peroxiredoxin 6 (PRDX6), prohormone convertase 2 (PCSK2), protein disulphide isomerase A6 (PDIA6), superoxide dismutase (SOD1), tubulin alpha (TUBA), tubulin beta 5 (TUBB5) and ATPase, H^+ transporting, lysosomal 70 M_r, V1 subunit A, isoform 1 (ATP6V1A)

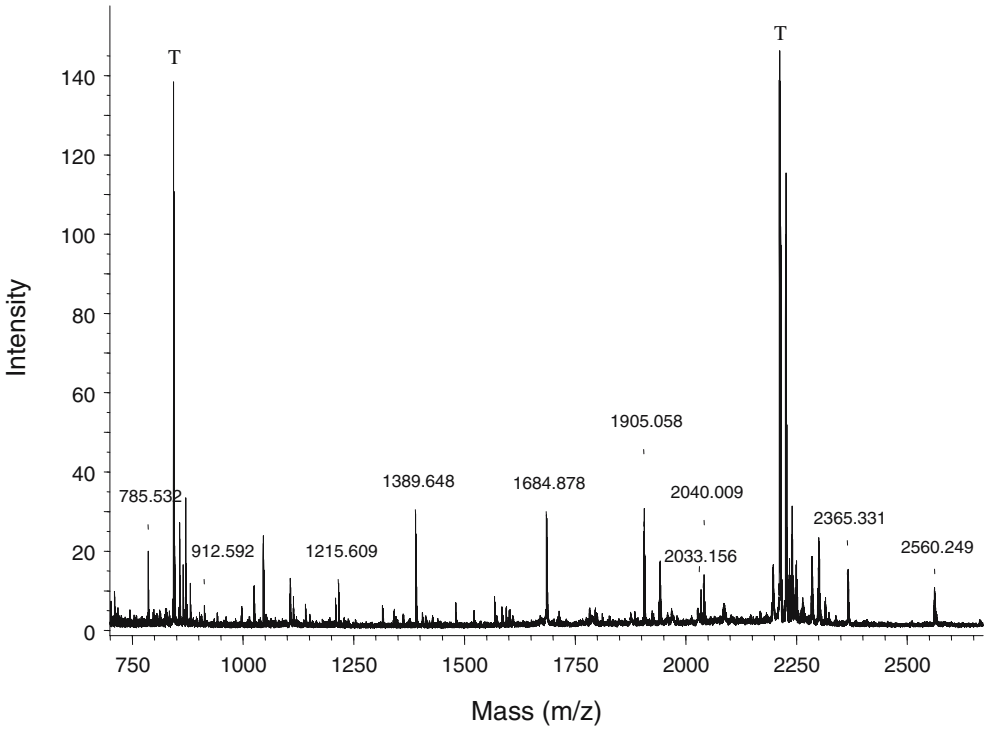

Fig. 2 Representative MALDI-TOF peptide mass spectrum of the 60-M_r heat shock protein, mitochondrial precursor (HSP60). The "T" denotes trypsin autodigestion fragments

(Fig. 4). Two proteins, which were represented by four spots, were present in gels of islets exposed to 11 mmol/l glucose but absent in fresh ones. These proteins were keratin, type II cytoskeletal 8 (KRT8) and BSA (Fig. 4). While the former is a component of the kinesin complex, the latter represents a component of the culture media. Proteins with less than 0.5-fold change in level, when islets exposed to 11 mmol/l glucose for 24 h were compared with freshly isolated islets, included 78-M_r glucose-regulated protein (GRP78), phosphatidylethanolamine-binding protein (PEBP), protein disulphide isomerase (PDI), secretagogin (SCGN) and the exocrine pancreas enzymes alpha-amylase (AMY2), carboxypeptidase B1 (CPB1), carboxypeptidase A1 precursor (CPA1), elastase IIIB precursor (ELA3B) and pancreatic triacylglycerol lipase (PNLIP) (Fig. 4). The exocrine pancreas enzymes were also measured in analytical gels (n=3) prepared from approximately 200 islets cultured at 11 mmol/l glucose for 7 days (Table 2). The extended culture period further decreased the levels of the enzymes.

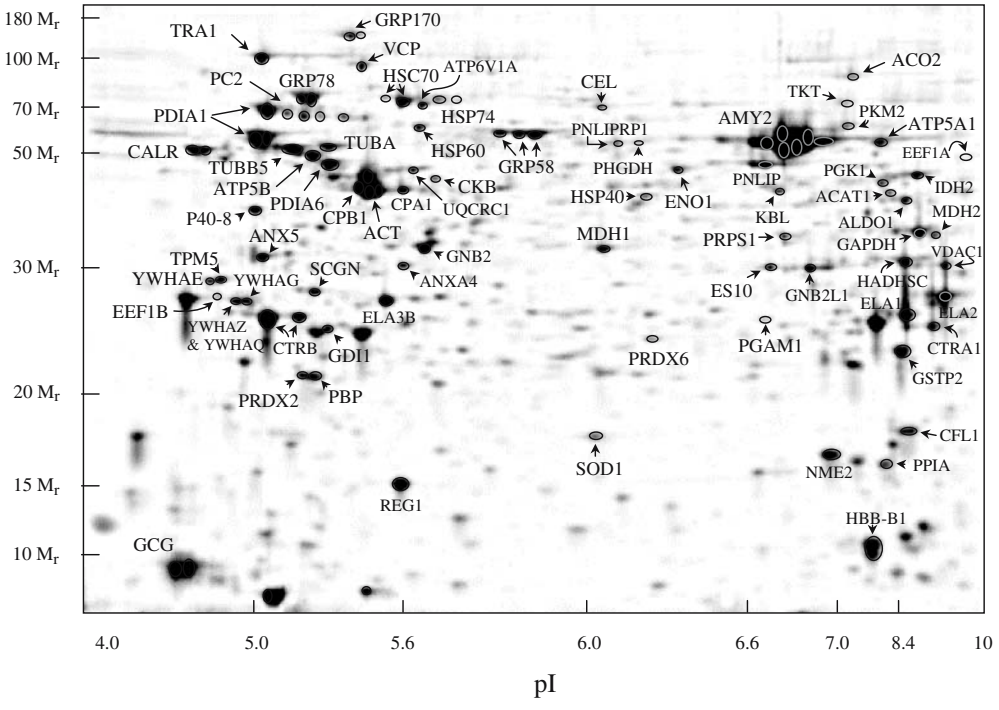

Fig. 3 The mouse islet reference map showing proteins obtained from 600 C57BL/6J islets, which contains approximately 500 μg of protein. The protein sample was loaded onto an IPG strip (pH 3–10 NL) and subsequently separated by mass on a gradient (8–16%) SDS-PAGE gel. The gel was stained with SYPRO Ruby and the filtered image was generated by PDQuest software. Experimental masses and p*I*s are indicated. The spots were analysed by MALDI-TOF MS and identified spots are designated with their gene acronyms

Table 1 Differentially expressed proteins in cultured mouse islets vs freshly isolated mouse islets

Gene name	Protein name	Freshly isolated islets	Cultured islets (1 day)	Ratio (culture 1 day/freshly isolated)
PDIA1	Protein disulphide isomerase	7,880	36	0.00
PDIA1	Protein disulphide isomerase	2,349	356	0.15
ELA3B	Elastase IIIB precursor	6,193	1,235	0.20
PNLIP	Triacylglycerol lipase, pancreatic	7,859	1,834	0.23
CPB1	Carboxypeptidase B1	14,009	3,681	0.26
CPA1	Carboxypeptidase A1 precursor	6,265	2,454	0.39
AMY2	Alpha-amylase, pancreatic	82,175	34,308	0.41
SCGN	Secretagogin	4,044	1,686	0.42
GRP78	78-M_r glucose-regulated protein precursor	23,058	10,427	0.45
PEBP	Phosphatidylethanolamine-binding protein	8,809	4,157	0.47
PCSK2	Prohormone convertase 2	487	1,029	2.11
PRDX6	Peroxiredoxin 6	1,079	2,335	2.16
PCSK2	Prohormone convertase 2	475	1,031	2.17
GRP58	58-M_r glucose-regulated protein	665	1,581	2.38
HSP40	Heat shock protein 40 M_r	1,542	3,791	2.46
ENO1	Alpha enolase	2,215	5,903	2.66
TUBB5	Tubulin, beta 5	6,568	17,874	2.72
TRA1	Endoplasmin	7,899	23,158	2.93
PCSK2	Prohormone convertase 2	241	721	2.99
HSP74	Heat shock protein cognate 74	273	821	3.01
HSP74	Heat shock protein cognate 74	201	659	3.28
ACT	Actin	6,105	20,326	3.33
PDIA6	Protein disulphide isomerase A6	711	3,013	4.24
SOD1	Superoxide dismutase (Cu–Zn)	1,218	5,222	4.29
PCSK2	Prohormone convertase 2	161	812	5.02
TUBA	Tubulin, alpha	264	1,622	6.14
ATP6V1A	ATPase, H^+ transporting, lysosomal 70-M_r V1 subunit A, isoform 1	505	3,804	7.52
KRT8	Keratin, type II cytoskeletal 8	138	1,491	10.74

Islets isolated from C57BL/6J mice were subjected to 2-DGE either directly after isolation or after 24 h culture. Approximately 200 islets were used for each gel. After staining, the spot densities of the proteins and the ratios between cultured and freshly isolated islets were determined

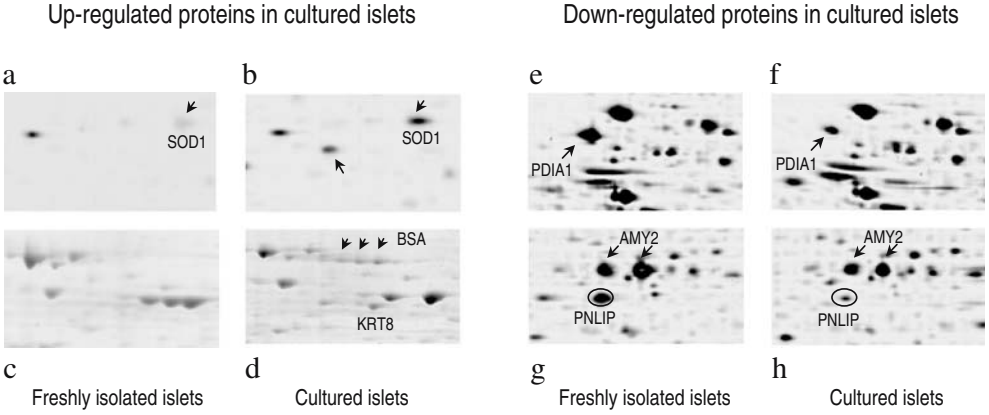

Fig. 4 Differentially expressed mouse islet proteins separated by 2-DGE. The *left panel* shows upregulated proteins in islets exposed to 11 mmol/l glucose. Variation in the quantity of superoxide dismutase (*SOD1*) is displayed in **a** and **b**, and appearance of a new spot (*arrow*) in the 2-DE gel of islets exposed to 11 mmol/l glucose (**c**). Keratin, type II cytoskeletal 8 (*KRT8*) and three spots (*arrows*), which were all identified as BSA, appeared as new spots in 2-D gels of islets exposed to 11 mmol/l glucose (**d**). The *right panel* shows proteins that are downregulated in islets exposed to 11 mmol/l glucose. Variation of protein disulphide isomerase (*PDIA1*) is shown in **e** and **f**, and changes in expression of proteins pancreatic alpha-amylase (*AMY2*) and pancreatic triacylglycerol lipase (*PNLIP*) in freshly isolated and islets exposed to 11 mmol/l glucose are displayed in **g** and **h**

Table 2 Differentially expressed pancreatic exocrine enzymes in islets cultured for 1 and 7 days

Gene name	Protein name	Ratio	
		Culture 1 day/ freshly isolated	Culture 7 days/ freshly isolated
ELA3B	Elastase IIIB precursor	0.20	0.03
PNLIP	Triacylglycerol lipase	0.23	0.03
CPB1	Carboxypeptidase B1	0.26	0.09
CPA1	Carboxypeptidase A1 precursor	0.39	[a]
AMY2	Alpha-amylase	0.41	0.02
PNLIPRP1	Pancreatic-lipase-related protein 1	0.64	0.03
CEL	Bile-salt-activated lipase precursor	0.64	0.12
CTRA1	Chymotrypsin A	[b]	[b]
ELA1	Elastase 1	[b]	[b]
ELA2	Elastase 2	[b]	[b]
CTRB1	Chymotrypsinogen B precursor	[c]	[c]

Islets isolated from C57BL/6J mice were subjected to 2-DGE either directly after isolation, after 24 h of culture or after 7 days of culture. Approximately 200 islets were used for each gel. After staining, the spot densities of the pancreatic exocrine enzymes and the ratios between cultured and freshly isolated islets were determined.

[a]Spot densities below detection, which makes it difficult to securely determine amounts

[b]Spots in basic portion of gel, which makes accurate determination difficult in multiple gels

[c]Spots in portion of the gel with many spots close to each other; reliable determination would require to narrow the pH range of the gel to ensure definite localisation in multiple gels

Discussion

In the present study the protein patterns of freshly isolated islets from fed control C57BL/6J mice were compared with protein patterns of islets exposed to 11 mmol/l glucose for 24 h. Previous work has shown that islet GSIS is influenced by prior exposure of the islets to glucose in a time- and concentration-dependent manner [8, 18, 19]. The importance of many specific proteins in relation to glucose regulation of islet secretory function has been described [9–11], but much is still unclear. This is partly explained by the fact that it is difficult to investigate a complex process like glucose-induced regulation of islet secretory function by studying one or a few parameters, which is the standard methodological format of most studies. It is therefore important, when new hypotheses about the regulation of glucose on islet function are to be generated, that the complexity of the many interacting islet proteins is taken into account. Proteomics is an approach that allows the generation of global protein expression profiles, and is therefore appropriate when such multifactorial processes are to be molecularly dissected. In the present study we have investigated glucose-regulated changes of the isolated islet by 2-DGE, which allows simultaneous measurement of multiple islet proteins.

When islet 2-D gels are prepared for comparative proteomic studies, a high-quality 2-DGE islet protein reference map facilitates the identification of differentially expressed proteins. The construction of the mouse islet reference map was initiated by the identification of 44 proteins [15]. Another 76 proteins were later added to the list [16, 17]. The current study, where approximately 1,300 spots were detected and 77 protein identities determined by MALDI-TOF analysis, adds 28 protein entries to proteins identified in mouse islets by proteome analysis. When a reference map is generated the source of the tissue has to be chosen carefully. Optimally, a reference gel should map the in vivo protein distribution under well-defined conditions. By choosing freshly isolated islets from fed male C57BL/6J mice as the source for our reference map, the islets were as close in time from the specified in vivo conditions as possible. However, effects of the isolation procedure may obviously distort the protein distribution. With the mouse islet reference map the identities of differentially expressed proteins in islets exposed to elevated glucose compared with in freshly isolated islets were determined. In a similar way differentially expressed rat islet proteins after exposure of the islets to interleukins have been identified successfully [20].

An important aspect of exposing islets to high glucose concentrations is that insulin biosynthesis is enhanced [21]. This aspect was reflected in our study by upregulation of 58 M_r glucose-regulated protein (GRP58), endoplasmin or 94 M_r glucose-regulated protein (TRA1), 170 M_r glucose-regulated protein (GRP170) and protein disulphide isomerase A6 (PDIA6) in islets exposed to elevated glucose concentration. These proteins are molecular chaperones involved in protein biosynthesis [22]. For an increase in insulin biosynthesis to manifest itself as a rise in GSIS, translocation of insulin granules from the Golgi region to the plasma membrane and maturation of granules are required. The former aspect was reflected in our study by upregulation of actin (ACT), tubulin beta 5 (TUBB5), tubulin alpha (TUBA) and keratin, type II cytoskeletal 8 (KRT8) and the latter aspect by upregulation of ATPase, H^+ transporting, lysosomal 70 M_r, V1 subunit A, isoform 1 (ATP6V1A) and prohormone convertase 2 (PCSK2) in islets exposed to elevated glucose concentration. In the case of prohormone convertase 2 (PCSK2), four spots were upregulated and three unchanged in islets exposed to elevated glucose compared with freshly isolated islets, which may reflect glucose-regulated post-translational modifications. In separate studies, actin, tubulin, related kinesins and probably also cytokeratins have been shown to be important for transport of insulin granules from the storage pool to the readily releasable pool [23–25]. The ATPase, H^+ transporting, lysosomal 70 M_r, V1 subunit A, isoform 1 (ATP6V1A) causes intragranular acidification, which is a prerequisite for proinsulin cleavage by the endopeptidases [26], but also for granular release competence [27].

Exposure of islets to elevated glucose concentration also induces stress responses. This aspect was reflected in our study by upregulation of superoxide dismutase (SOD1), peroxiredoxin 2 (PRDX2) and 6 (PRDX6), heat shock protein cognate 74 (HSP74) and heat shock protein 40 M_r (HSP40). While superoxide dismutase (SOD1) and the peroxiredoxins (PRDX2 and PRDX6) will prevent damage caused by enhanced production of reactive oxygen species by elevated glucose [28–30], the heat shock proteins (HSP74 and HSP40) are instrumental for maintenance of organelle function [31].

An established effect of keeping isolated islets in culture is that any remnants of exocrine cells decrease with time. The finding of exocrine proteins in the 2-D protein gels of freshly isolated islets indicates presence of acinar cells. However, the quantities of the exocrine proteins decreased in islets kept under culture conditions for 24 h compared with the freshly isolated islets and were virtually absent in a 2-D protein gel of islets cultured for 7 days. Since acinar tissue has detrimental effects on insulin release [32], it is possible that gradual loss of acinar cells in cultured islets could contribute to the enhanced glucose responsiveness observed in the cultured islets.

In conclusion, using 2-DGE and MS we have obtained information about changes in expression of multiple islet proteins in response to elevation of glucose concentrations. The low number of identified proteins in the present study does not allow strict differentiation of the effects of high glucose on islet protein expression in cultured islets vs expression in freshly isolated islets. However, the study shows that by combining such different protein expressions, complex biological processes can be elucidated.

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