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Proteome analysis of human stomach tissue: Separation of soluble proteins by two-dimensional polyacrylamide gel electrophoresis and identification by mass spectrometry

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Summary

Two-dimensional gel electrophoresis (2-DE) maps for human stomach tissue proteins have been prepared by displaying the protein components of the tissue by 2-DE and identifying them using mass spectrometry. This will enable us to present an overview of the proteins expressed in human stomach tissues and lays the basis for subsequent comparative proteome analysis studies with gastric diseases such as gastric cancer. In this study, 2-DE maps of soluble fraction proteins were prepared on two gel images with partially overlapping pH ranges of 4-7 and 6-9. On the gels covering pH 4-7 and pH 6-9, about 900 and 600 protein spots were detected on silver staining, respectively. For protein identification, proteins spots on micropreparative gels stained by colloidal Coomassie Brilliant Blue G-250 were excised, digested in-gel with trypsin, and analyzed by peptide mass fingerprinting with delayed extraction-matrix assisted laser desorption/ionization-mass spectrometry (DE-MALDI-MS). In all, 243 protein spots (168 spots in acidic map and 75 spots in basic map) corresponding

to 136 different proteins were identified. Besides these principal maps, maps of lower resolution, i.e. overview maps (displayed on pH 3-10 gels) for total homogenate and soluble fraction, are also presented with some identifications mapped on them. Based on the 2-DE maps presented in this study, a 2-DE database for human stomach tissue proteome has been constructed and available at http://proteome.gsnu.ac.kr/DB/2DPAGE/Stomach/. The 2-DE maps and the database resulting from this study will serve important resources for subsequent proteomic studies for analyzing the normal protein variability in healthy tissues and specific protein variations in diseased tissues.

1. Introduction

Proteomics aims at the global analysis of tissue and cellular proteins, and uses a combination of techniques including two-dimensional gel electrophoresis (2-DE), image analysis, mass spectrometry, and bio-informatics to resolve comprehensively, to quantify, and to characterize proteins. With the rapid development in proteomics technologies and a great deal of current interest in the application of proteomics to the study of various human diseases, our knowledge of how protein profiles vary in healthy and diseased tissues is accruing at an impressive pace [1-3]. Recently, the proteomics approach is being actively applied to the molecular analysis of various human cancers such as bladder [4], colorectal [5,6], breast [7], and liver [8] cancers. Gastric cancer is the most common cancer in Korea and other Asian countries. Although incidence rates in western world are much lower than in Asia, gastric carcinoma is still a significant worldwide health burden, second only to lung tumors as a leading cause of cancer deaths. To initiate a gastric cancer proteomics project, we thought 2-DE maps are an important prerequisite as the separation and

identification of proteins in normal tissues are important steps in establishing a suitable database of identifiable proteins and subsequently applying proteomic analyses to pathological perturbations. Also, 2-DE is the principal step of proteomics, and is widely used in comparative studies of protein expression levels between healthy and diseased states with the purpose of developing diagnostic markers and detecting novel drug targets. Hence, the present study was designed to explore the use of 2-DE for separating human stomach tissue proteins and to initiate the construction of 2-DE maps and a database of identified proteins. This database should provide a valuable resource for the investigation of the molecular basis of gastric cancer and also other human stomach pathologies.

2. Materials and methods

2.1 Materials

IPG strips of pH 3-10, 4-7, and 6-9 were purchased from Amersham Pharmacia Biotech (APB, Immobiline DryStrip, 0.5×3×180 mm) and Bio-Rad (ReadyStrip, 0.5×3×170 mm). Bio-Lyte (pH 3-10) was from Bio-Rad. SDS, acylamide, methylenebisacrlamide, TEMED, ammonium persulfate, DTT, urea, Tris, glycine, glycerol and CHAPS were purchased from Bio-Rad or USB. Silver nitrate, Coomassie brilliant blue G-250, TCA, iodoacetamide, and ¥á-cyano-4-hydroxycinnamic acid were from Sigma. Methanol, ethanol, phosphoric acid, acetic acid and formaldehyde were purchased from Merck. Sequencing grade modified trypsin was obtained from Promega. Other reagents were obtained from Sigma or Merck.

2.2 Stomach tissue samples

Human stomach tissue samples were prepared from resection materials of

gastric cancer patients in Gyeongsang National University Hospital. Resections were examined by a pathologist and normal tissue samples were prepared from noncancerous regions. Four tissue samples were used in this study: two samples, no. 299371 and 232285, were used to construct the 2-DE maps; and the other two, no. 368412 and 000925, were used for micropreparative gels for protein identification.

2.3 Preparation of stomach tissue protein samples

Frozen stomach tissue samples (100-200 mg) were homogenized in 2 mL homogenization buffer (50 mM Tris-HCl, pH 7.2) containing protease inhibitor cocktail (1 mM AEBSF, 0.8 μM aprotinin, 21 μM leupeptin, 36 μM bestatin, 15 μM pepstatin A, 14 μM E-64) using Utra-Turrax homogenizer (type T8, IKA Labortechnik, Germany) at 25,000 rpm. The mixture was centrifuged at 1000×g for 5 min to remove tissue and cell debris. The supernatant was used as total homogenate. The total homogenate was centrifuged in a Beckman TL-100 table top ultracentrifuge at 100,000 rpm (approx. 430,000×g) in a TLA-100.2 rotor for 10 min at 4°C. The supernatant was taken as soluble fraction and the pellet was used for membrane protein preparation. 50 %(w/v) TCA was added to the supernatant to a final concentration of 10 %(w/v) and the solution was allowed to stand on ice for 30 min. Protein precipitate was collected by spin in a microcentrifuge at 15,000 rpm for 10 min at 4°C, and washed three times in 10 % TCA. The precipitate was washed once in diethyl ether and dried under air stream. The dry pellet was dissolved with sonication in the lysis solution (8 M urea, 4 %(w/v) CHAPS, 40 mM Tris, 100 mM DTT, 2 %(w/v) Bio-Lyte (pH 3-10)) and allowed to stand for 1 h at room temperature. After centrifugation at 15,000 rpm for 10 min at 15°C, the supernatant was used as the 2-DE sample for soluble fraction. The protein samples were stored in aliquots at -70°C until

use. For the preparation of membrane fraction, the ultracentrifugal pellet was washed once in PBS, dissolved in the lysis solution with sonication, centrifuged at 15000×g for 10 min at 15°C, and the supernatant was used as membrane fraction sample.

2.4 Protein Assay

Protein concentration of 2-DE samples was estimated according to [9] using a commercial Bradford reagent (Bio-Rad). BSA was used as standard.

2.5 2-DE

IEF was carried out using commercially available, dedicated apparatuses: IPGphor (Amersham Pharmacia Biotech) or Protean IEF Cell (Bio-Rad). IPG strips were used according to [10] and the manufacturers instructions. Samples containing up to 200 μg protein for analytical gels or up to 1 mg for micropreparative gels, were diluted to 300-350 μL with rehydration solution (8 M urea, 2% CHAPS, 100 mM DTT, 0.5% (v/v) pH 3-10 IPG buffer, trace bromophenol blue), and applied to strips by overnight rehydration at 50 V. Proteins were focused succeedingly for 1 h at 200 V, for 1 h at 500 V, for 1 h at 1000 V, then a gradient was applied from 1000 to 8000 V in 30 min, and focusing was continued at 8000V for 8.5 h to give a total of 70 kVh on an IPGphor. With Protean IEF Cell, focusing was done initially at 250 V for 15 min, then the voltage was increased to 10000 V within 3 h, and maintained at 10000 V for 7 h for a total of 70 kVh. All IEF steps were carried out at 20°C. After the first-dimensional IEF, IPG gel strips were placed in an equilibration solution (6 M urea, 2% SDS, 30% glycerol, 50 mM Tris-HCl, pH 8.8) containing 1% DTT for 10 min with shaking at 50 rpm on an orbital shaker. The gels were then transferred to the equilibration solution containing 2.5% iodoacetamide and

shaken for a further 10 min before placing them on a 7.5-17.5% gradient polyacrylamide gel slab (185×200×1.0 mm). Separation in the second dimension was carried out using Protean II xi electrophoresis equipment and Tris-glycine buffer (25 mM Tris, 192 mM glycine) containing 0.1% SDS, at a current setting of 5 mA/gel for the initial 1 h and 10 mA/gel thereafter. The second dimensional SDS-PAGE was developed until the bromophenol blue dye marker had reached the bottom of the gel.

2.6 Protein visualization and image analysis

For silver staining, following second-dimensional SDS-PAGE, analytical gels were immersed in methanol: acetic acid: water (50:12:38) for 1.5 h, followed by washing twice in 50% ethanol for 20 min. Gels were pretreated for 1 min in a solution of 0.02% $Na_2S_2O_3$. This was followed by three 1 min washes in deionized water. Proteins were stained in a solution containing 0.2% $AgNO_3$ and 0.075% (v/v) formalin (37% formaldehyde in water) for 20 min, and washed twice in deionized water for 1 min. Subsequently, gels were developed in a solution of 0.06%(v/v) formalin, 2% Na_2CO_3, and 0.0004% $Na_2S_2O_3$. When the desired intensity was attained, the developer was discarded and stopped by 1% acetic acid.

For Coomassie blue staining of micropreparative gels, gels were fixed 3 times in 30% ethanol containing 2% phosphoric acid for 20 min and rinsed 3 times in 2% phosphoric acid. Gels were then equilibrated in a solution containing 18% ethanol, 2% phosphoric acid, and 15% ammonium sulfate for 30 min and Coomassie Brilliant Blue G-250 was added to a final concentration of 1%. Staining was carried out overnight.

Protein patterns in the gels were recorded as digitalized images using a high resolution scanner (GS-710 Calibrated Imaging Densitometer, Bio-Rad). Gel image matching was done with PDQuest software (Bio-Rad).

2.7 In-gel digestion

In-gel digestion of protein spots on Coomassie or silver stained gels was performed essentially as described by [11]. After the completion of staining, the gel slab was washed twice with water for 10min. The spots of interest were excised with a scalpel, cut into pieces, and put into 1.5-mL microtubes. The particles were washed twice with water for 15 min, and then twice with water/acetonitrile (1:1, v/v) for 15 min. The solvent volumes were about twice the gel volume. Liquid was removed, acetonitrile was added to the gel particles and the mixture was left for 5 min. Liquid was removed and the particles were rehydrated in 0.1 M NH_4HCO_3 for 5 min. Acetonitrile was added to give a 1:1 (v/v) mixture of 0.1 M NH_4HCO_3/acetonitrile and the mixture was incubated for 15 min. All liquid was removed and gel particles were dried in a vacuum centrifuge (Heto-Holten, Allerød, Denmark), reswelled in 10 mM DTT/0.1 M NH_4HCO_3, and incubated for 45 min at 56°C to reduce the peptides. After chilling tubes to room temperature and removing the liquid, 55 mM iodoacetamide in 0.1 M NH_4HCO_3 was added, the tubes were incubated for 30 min at room temperature in the dark to S-alkylate the peptides. Iodoacetamide solution was removed, the gel particles were washed with 0.1 M NH_4HCO_3 and acetonitrile, dried in a vacuum centrifuge, rehydrated on ice in digestion buffer containing 50 mM NH_4HCO_3, 5 mM $CaCl_2$, and 12.5 ng/μL of trypsin, and incubated for 45 min on ice. Excess liquid was removed and about 20 μL of digestion buffer without trypsin was added. After overnight digestion at 37°C, 25 mM NH_4HCO_3 was added, and the tube was incubated for 15 min. Acetonitrile was added and the tube was incubated for a further 15 min. The supernatant was recovered, and the extraction was repeated twice with 5% formic acid/acetonitrile (1:1, v/v). The three extracts were pooled and dried in a vacuum centrifuge.

2.8 MALDI-TOF MS and database search

Tryptic peptides were redissolved in a solution containing water, acetonitrile, and trifluoroacetic acid (93:5:2 by volume), and the solution was treated for 5 min in a bath sonicator. Target preparation was carried out by 'solution phase nitrocellulose method' [12]. Saturated solution of α-cyano-4-hydroxycinnamic acid (about 40 mg/ml) and nitrocellulose solution (20 mg/ml) were prepared separately in acetone. A mixture of the α-cyano-4-hydroxycinnamic acid solution, nitrocellulose solution, and 2-propanol was prepared at a ratio of 2:1:1. Peptide calibrants (50-200 fmole of each), des-Arg-bradykinin (monoisotopic mass, 904.4681) and neurotensin (1672.9715), were added and the mixture solution was then spotted on the target and dried. Dried samples were washed with 5 μL of 5% formic acid for 10 s, followed by 5 μl of Milli-Q water for 10 s, and then dried spots were analyzed in a Voyager-DE STR MALDI-TOF mass spectrometer (PerSeptive Biosystems, Framingham, NA, USA). The spectrometer was run in positive ion mode and in reflector mode with the settings: accelerating voltage, 20 kV; grid voltage, 76%; guide wire voltage, 0.01%; and a delay of 150 ns. The low mass gate was set at 500 m/z. Proteins were identified by peptide mass fingerprinting with the search programs MS-FIT (http://prospector.ucsf.edu/ucsfhtml3.4/msfit.htm) and ProFound (http://129.85.19.192/profound_bin/WebProFound.exe). The following search parameters were applied: SWISS-PROT and NCBI were used as the protein sequence databases; a mass tolerance of 50 ppm and one incomplete cleavage were allowed; acetylation of the N-terminus, alkylation of cysteine by carbamidomethylation, oxidation of methionine, and pyroGlu formation of N-terminal Gln were considered as possible modifications.

3. Results and discussion

The establishment of 2-DE protein map is a prerequisite for subsequent

proteomic studies of a given biological system. It is an urgent issue to many Asian countries to apply post-genomic approaches including proteomics to gastric diseases such as gastric cancer, as they are among the most prevalent diseases in the area. However, there has been no 2-DE map reported for human stomach tissue proteins.

3.1. Conception of 2-DE maps for human stomach tissue proteome

Reproducibility of 2-DE has improved significantly since the introduction of IPG focusing technology [13]. Nevertheless, it is still not an easy job to compare gels of different resolution. For example, when one is analyzing a sample with pH 4-7 strips and finds an interesting spot on 2-D gel, it is usually hard to identify one's protein by image matching with a 2-DE map of different resolution available on an internet site such as Swiss-2DPAGE (http://www.expasy.ch/ch2d/) or WORLD 2DPAGE (http://www.expasy.ch/ch2d/2d-index.html) databases. Thus, for a given sample, it is important to construct maps that cover different levels of resolution. To construct 2-DE maps of human stomach tissue that would be useful for wide spectrum of researches on gastric diseases, we have conceived multiple level maps (Fig. 1). The lowest resolution and most comprehensive map, which we call level 1 map, is prepared with the total homogenate of stomach tissue separated by wide pH range (3-10) focusing. At level 2 resolution, total homogenate is fractionated into soluble and membrane fractions, which are separated on pH 3-10 IPG strips. The maps of level 1 and level 2 constitute overview maps. At level 3, each of the soluble and membrane fractions is further resolved on two partially overlapping medium pH range (4-7 and 6-9) strips, which provides maps of intermediate level of resolution. The highest level of resolution that can be obtained with commercially available IPG strips is one using 1 pH unit-wide strips [14,15], and even higher resolution

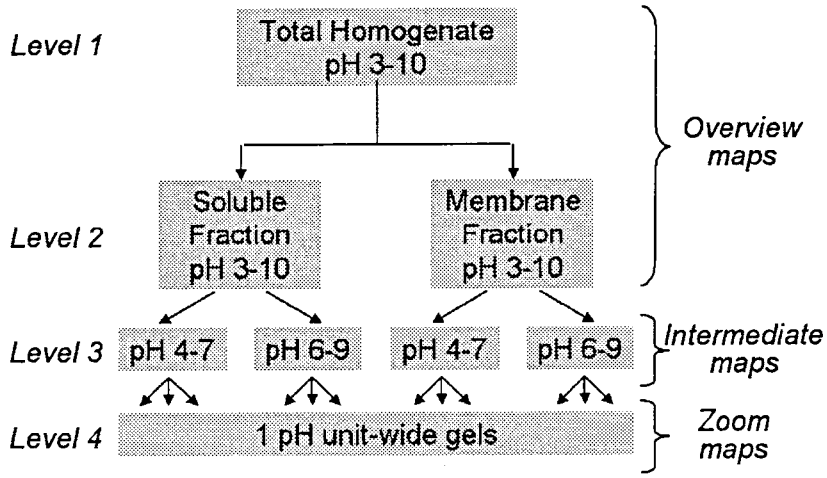

Figure 1. Conception of 2-DE maps for human stomach tissue proteins. The most comprehensive and lowest resolution map, level 1 map, is prepared with total homogenate of stomach tissue separated on wide pH range (3-10) focusing. At level 2 resolution, total homogenate is fractionated into soluble and membrane fractions, which are separated on pH 3-10 IPG strips. The level 1 and level 2 maps present an overview on the global composition of major proteins in the total homogenate and each fraction. At level 3, each of the soluble and membrane fractions is further resolved on two partially overlapping medium pH range (4-7 and 6-9) gels, thus providing an intermediate resolution map. Strips of 1 pH unit-wide range can be used in the first dimension to prepare level 4 or zoom maps.

would be possible by using home-made sub-unit pH range strips and larger format gels [16,17]. Also, resolution can be increased at all levels by appropriate pre-fractionations of protein samples. In this study, analyses of clinical samples were kept in mind as a main application of proteomics. Because of the heterogeneous nature of clinical samples, it is important in clinical proteomics to analyze certain number of samples that can guarantee statistical significance of observed change in expression. Thus, as a compromise between high resolution and practicability, level 3 maps consisting of four images (acidic soluble, basic

soluble, acidic membrane, and basic membrane protein maps) from 200 mm-wide gels using 170 or 180 mm strips were conceived as the main maps of human stomach tissue proteome, and overview maps of level 1 and 2 were considered as supplementary.

3.2. Human stomach tissue 2-DE maps of soluble proteins

A series of 2-DE maps covering several levels of resolution was constructed for the soluble fraction proteins of human stomach tissue. All maps (Fig. 2 and Fig. 3) were constructed on silver stained analytical gels, with protein loadings of 200 μg.

Stomach tissue samples were obtained from healthy regions of gastric resections of gastric cancer patients. Frozen tissue samples were mechanically homogenized and tissue debris were removed by low speed centrifugation to give a total homogenate. The total homogenate proteins were precipitated with 10% TCA and ether-washed pellet was extracted with mild sonication into lysis solution containing 8 M urea, 4%(w/v) CHAPS, 40 mM Tris, 100 mM DTT and 2% (w/v) Bio-Lyte (pH 3-10). Fig. 2A shows the level 1 map of human stomach tissue. The initial severe streaking could be reduced significantly by using TCA precipitated protein samples. About 1400 spots could be detected on the gel by the Auto-Detect Spots menu of PDQuest software. Good resolutions were obtained in acidic and neutral pH regions, while streaks were observed in basic proteins and some abundant protein spots such as serum albumin (P02768) and actin (P02570). The abundance of serum albumin is typical of frozen tissues and is due to blood contamination in the tissue.

To construct a 2-DE map, it is important to have a representative sample. It is relatively straightforward with microorganisms and cell lines, where genetically and physiologically homogeneous cell populations can be easily

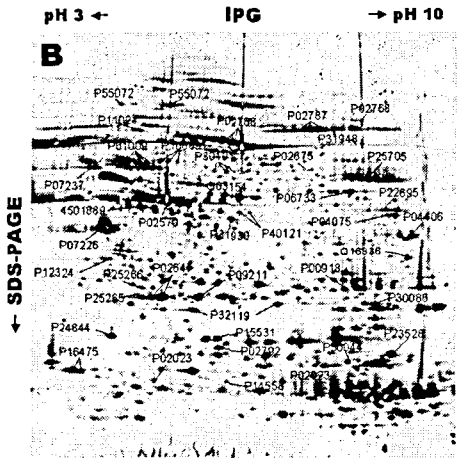

Figure 2. Overview 2-DE maps of human stomach tissue. (A), Total homogenate; (B), Soluble fraction; (C), Membrane fraction. Proteins (100 μg) were separated on pH 3-10 nonlinear IPG strip in the first dimension and 7.5-17.5% linear gradient SDS-PAGE in the second dimension. Staining was done with silver nitrate. A total homogenate of stomach tissue was obtained by mechanical homogenization and removal of tissue debris by low speed centrifugation, and TCA precipitated proteins were extracted into lysis solution. Soluble and membrane fractions were prepared from the total homogenate by ultracentrifugation. Spot identifications (Swiss-Prot accession numbers) were transferred from the maps of Fig. 3 by image matching. The protein names are listed in Table 1.

obtained and used for preparation of protein samples. However, it may be complicated with human tissues, such as stomach tissue of this study, where variations in protein profile might occur for different locations on stomach and also from genetic heterogeneity among different individuals. One way to cope with such a potential heterogeneity is to obtain an average profile that involves the pooling of a certain number of samples [18]. In the preparation of 2-DE maps presented in this study, tissue samples from two individuals were used without pooling of samples: one tissue for the overview maps of Fig. 2, and the

other for the intermediate maps of Fig. 3, as our initial analyses of ten normal tissue samples on 180 mm strips of pH 4-7 linear gradient indicated the overall protein pattern remained very similar across samples (not shown).

The total homogenate was fractionated by ultracentrifugation into soluble (supernatant) fraction and membrane (pellet) fraction. 100 μg proteins of each fraction were separated on pH 3-10 nonlinear gradient strips and SDS-PAGE. The protein profiles are shown in Fig. 2B and 2C, respectively, for soluble and membrane fractions. These wide pH range gels give overviews on protein profile of each fraction. About 1300 and 1500 spots were detected, respectively, on gels for soluble and membrane fraction. The membrane protein maps will be the subject of a separate study.

The soluble fraction proteins (200 μg) were further separated on two medium pH range gels of 4-7 and 6-9, and stained with silver nitrate. These two level 3 maps (Fig. 3A and 3B) constitute the main maps for the soluble proteins of human stomach tissue. The acidic map (Fig. 3A) contained about 900 spots and the basic one (Fig. 3B) about 600 spots. For construction of the level 3 maps, both Coomassie stained micropreparative gels and silver stained analytical gels were used. Though most spot identifications were done from Coomassie stained gels, some spots were more clearly detected on and analyzed from silver stained gels. Micropreparative gels (not shown) were run with a loading of 500 to 1000 μg proteins per gel, and stained with colloidal Coomassie Brilliant Blue G-250. After matching micropreparative gel image with analytical one by use of PDQuest, spots were cut out from the micropreparative gel and processed for MALDI-TOF mass spectrometry as described in Materials and methods. The resulting spot identification was mapped onto the analytical gels as shown in Fig. 3A and 3B. In the acidic map, 588 spots were excised and subjected to in-gel digestion followed by peptide mass fingerprinting for protein identification.

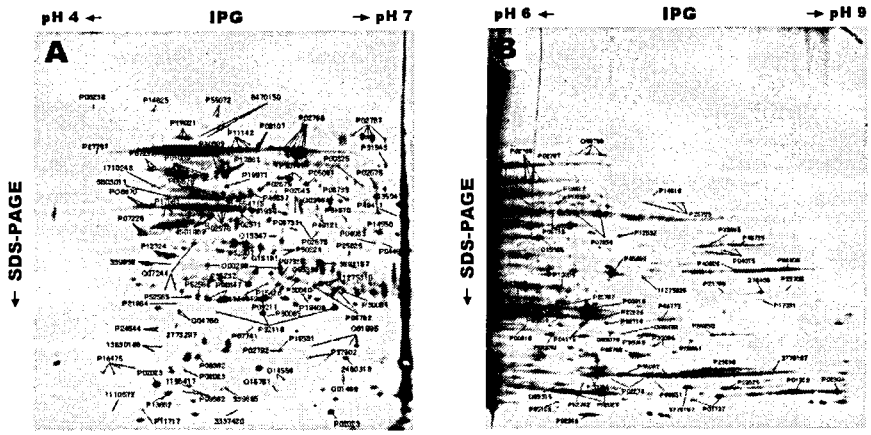

Figure 3. Medium resolution (at level 3) 2-DE maps of soluble fraction proteins of human stomach tissue. The soluble fraction was prepared as described in the legend of Figure 3. The proteins were displayed on two gels of overlapping pH range of 4-7 (A) and 6-9 (B). Two hundred micrograms of proteins were loaded onto each gel and staining was done with silver nitrate. Protein identifications were done on corresponding preparative gels (not shown) stained with colloidal Coomassie Brilliant Blue G-250 and transferred onto analytical gels by image matching. All labeled spots have been identified by peptide mass fingerprinting. Identified spots are indicated by their accession numbers.

168 spots corresponding to 92 different proteins were successfully identified. In the basic map, 75 spots corresponding to 49 proteins were identified out of 157 spots analyzed. 21 spots of 5 proteins were identified on both gels. Overall, 745 spots were analyzed and 243 spots corresponding to 136 proteins of human stomach tissue have been identified and mapped on two gel images of Fig. 3A and 3B. On average, each protein was represented by 1.7 spots. 53 proteins (39% of 136 proteins) were present in multiple spots: 30 proteins in 2 spots, 9 in 3, 7 in 4; serrotransferrin, deskman, alpha-1-antitrypsin, and serum albumin were present in 5-7 spots. Most of these multiple spots appeared in so-called trains, i.e. a series of nearly horizontal spots in regular intervals. However, as in the

case of peroxiredoxin 2 (Q06830), some proteins appeared in two spots that are far apart on the gel. Of the 136 identified proteins: 40 proteins were cytoplasmic, 23 mitochondrial, 9 nuclear, 8 cytoplasmic and nuclear, 6 ER lumen, 2 lysosomal, 1 peroxisomal proteins; 6 proteins were extracellular; and 40 proteins were of uncertain location. Thus, most proteins, for which information on subcellular location was available, were extracellular, cytoplasmic, or from the inner space of subcellular organelles such as mitochondria and endoplasmic reticulum. This corroborates with the fact that the proteins displayed on the two gels were soluble fraction proteins and thus of hydrophilic nature. The result indicates also that subcellular organelles are disrupted to a considerable extent during the mechanical homogenization process, because 30% of the total identified proteins were from organelles.

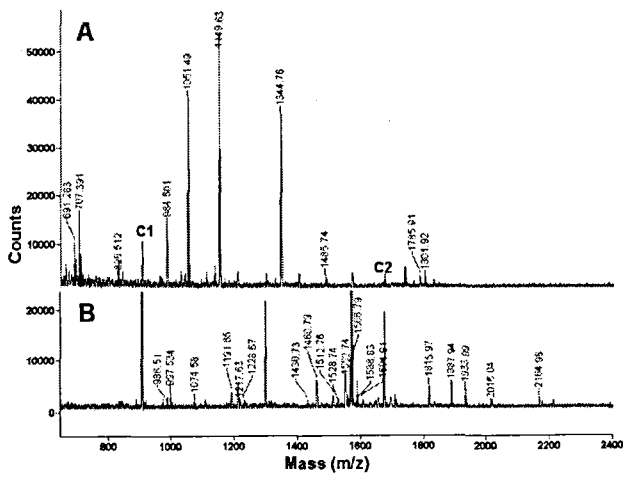

Figure 4. MALDI-TOF mass spectra of tryptic digests of two protein spots resolved on 2-DE gel. (A), Spot 5134; matched peptides, 10; amino acid coverage, 53%; the spot was identified as nucleoside diphosphate kinase A (Swiss-Prot No., P15531). (B), Spot 2733; matched peptides, 19; amino acid coverage, 31%; 78 kDa glucose-regulated protein (Swiss-Prot. No., P11021). The data were collected on positive ion and reflector mode. Experimentally determined mass values are labeled only on matched peptides. Calibrants: C1, bradykinin fragment 2-9, monoisotopic mass 904.4681; C2, neurotensin, 1672.9175.

Table 1. Proteins identified in the human stomach tissue.

No.	Accession Number[a]	Entry name[b]	Protein	Subcellular Location[c]	*Mr* (Da)[d]	p*I*[d]	Match	Seq. cov. (%)	Freq. (n)[e]	Gel[f]
1	O00299	CLI1_HUMAN	Chloride intracellular channel protein 1	Nulcear	26924	5.09	9	44	2	A
2	O14558	HSBX_HUMAN	Heat-shock 20 kDa like-protein P20	-	16937	5.95	6	36	2	A
3	O95336	6PGL_HUMAN	6-Phosphogluconolactonase	Cytoplasmic	27547	5.70	8	34	1	A
4	P00325	ADHB_HUMAN	Alcohol dehydrogenase beta chain	Cytoplasmic	54731	6.29	5	17	1	A
5	P00367	DHE3_HUMAN	Glutamate dehydrogenase 1	Mitochondrial matrix.	61398	7.66	12	20	1	B
6	P00918	CAH2_HUMAN	Carbonic anhydrase II	Cytoplasmic	29246	6.87	11	47	2	B
7	P00938	TPIS_HUMAN	Triosephosphate isomerase	Cytoplasmic	26538	6.51	9	34	1	B
8	P01009	A1AT_HUMAN	Alpha-1-antitrypsin	Extracellular	46737	5.37	13	30	6	A
9	P01922	HBA_HUMAN	Hemoglobin alpha chain	Cytoplasmic	15258	8.72	5	42	1	B
10	P02023	HBB_HUMAN	Hemoglobin beta chain	Cytoplasmic	15867	6.81	7	53	4	A, B
11	P02248	UBIQ_HUMAN	Ubiquitin	Nuclear, Cytoplasmic	8565	6.56	4	50	1	B
12	P02278	H2BA_HUMAN	Histone H2B.a/g/k	Nuclear	13775	10.31	8	41	2	B
13	P02304	H4_HUMAN	Histone H4	Nuclear	11236	11.36	6	45	1	B
14	P02545	LAMA_HUMAN	Lamin A/C	Nuclear	74540	6.59	11	21	2	A
15	P02570	ACTB_HUMAN	Actin, cytoplasmic 1	Cytoplasmic	41737	5.29	9	30	4	A
16	P02571	ACTG_HUMAN	Actin, cytoplasmic 2	Cytoplasmic	41662	5.31	9	23	1	A
17	P02647	APA1_HUMAN	Apolipoprotein A-I	Extracellular	30778	5.56	14	52	2	A

Table 1. continued

No.	Accession Number	Entry name	Protein	Subcellular location	*Mr* (Da)	p*I*	Match	Seq. cov. (%)	Freq. (n)	Gel
18	P02675	FIBB_HUMAN	Fibrinogen beta chain	Extracellular	55928	8.54	12	29	3	A
19	P02679	FIBG_HUMAN	Fibrinogen gamma-A chain	Extracellular	49482	5.61	7	19	1	A
20	P02768	ALBU_HUMAN	Serum albumin	Extracellular	69367	5.92	19	34	7	A, B
22	P02792	FRIL_HUMAN	Ferritin light chain	Cytoplasmic	20020	5.51	5	36	1	A
23	P04075	ALFA_HUMAN	Fructose-bisphosphate aldolase A	Cytoplasmic	39420	8.30	9	31	3	B
24	P04083	ANX1_HUMAN	Annexin I	-	38715	6.57	11	41	1	A
25	P04179	SODM_HUMAN	Superoxide dismutase [Mn]	Mitochodrial matrix	26538	6.51	9	39	1	B
26	P04406	G3P2_HUMAN	Glyceraldehyde 3-phosphate dehydrogenase	Cytoplasmic	36054	8.57	8	30	1	A, B
27	P04792	HS27_HUMAN	Heat shock 27 kDa protein	Cytoplasmic	22327	7.83	9	42	2	A
28	P05091	DHAM_HUMAN	Aldehyde dehydrogenase	Mitochodrial matrix	56382	6.63	8	17	1	A
29	P05092	CYPH_HUMAN	Peptidyl-prolyl cis-trans isomerase A	Cytoplasmic	17881	7.82	9	45	1	C
30	P05109	S108_HUMAN	Calgranulin A	-	10835	6.51	5	43	1	C
31	P06576	ATPB_HUMAN	ATP synthase beta chain	Mitochodrial	56560	5.26	13	31	2	A
32	P06733	ENOA_HUMAN	Alpha enolase	Cytoplasmic	47038	6.99	16	41	4	A, B
33	P07226	TPM4_HUMAN	Tropomyosin, fibroblast non-muscle type	-	28522	4.67	8	21	2	A

Table 1. continued

No.	Accession Number	Entry name	Protein	Subcellular location	*Mr* (Da)	p*I*	Match	Seq. cov. (%)	Freq. (n)	Gel
34	P07237	PDI_HUMAN	Protein disulfide isomerase	ER lumen	55294	4.69	11	23	2	A
35	P07339	CATD_HUMAN	Cathepsin D	Lysosomal	44553	6.10	9	20	1	A
36	P07737	PRO1_HUMAN	Profilin I	Cytoplasmic	15054	8.44	6	35	2	B
37	P07741	APT_HUMAN	Adenine phosphoribosyltransferase	Cytoplasmic	19608	5.78	7	50	1	A
38	P07954	FUMH_HUMAN	Fumarate hydratase	Mitochodrial, Cytoplasmic	54637	8.85	9	22	2	B
39	P08107	HS71_HUMAN	Heat shock 70 kDa protein 1	Mitochodrial, ER	70052	5.48	13	23	2	A
40	P08238	HS9B_HUMAN	Heat shock protein HSP 90-beta	Cytoplasmic	83295	4.97	11	18	1	A
41	P08670	VIME_HUMAN	Vimentin	Cytoplasmic	53686	5.06	13	28	3	A
42	P09211	GTP_HUMAN	Glutathione S-transferase P	-	23356	5.43	10	55	1	A
43	P09382	LEG1_HUMAN	Galectin-1	-	14585	5.34	5	39	3	A
44	P09622	DLDH_HUMAN	Dihydrolipoamide dehydrogenase	Mitochodrial matrix	54151	5.39	8	18	1	B
45	P09651	ROA1_HUMAN	Heterogeneous nuclear ribonucleoprotein A1	Nuclear, Cytoplasmic	38715	9.26	9	42	1	B
46	P10809	CH60_HUMAN	60 kDa heat shock protein, mitochondrial	Mitochodrial matrix	61055	5.70	14	30	3	A
47	P11021	GR78_HUMAN	78 kDa glucose-regulated protein	ER lumen	72111	5.03	18	32	3	A
48	P11142	HS7C_HUMAN	Heat shock cognate 71 kDa protein	-	70898	5.38	12	21	4	A

Table 1. continued

No.	Accession Number	Entry name	Protein	Subcellular location	*Mr* (Da)	p*I*	Match	Seq. cov. (%)	Freq. (n)	Gel
49	P11717	MPRI_HUMAN	Cation-independent mannose-6-phosphate receptor	Lysosomal	17431	5.62	8	32	1	A
50	P12324	TPMN_HUMAN	Tropomyosin, cytoskeletal type	Cytoplasmic	29033	4.75	14	41	3	A
51	P12532	KCRU_HUMAN	Creatine kinase, ubiquitous mitochondrial	Mitochondrial	47037	8.60	11	27	1	B
52	P13662	NTF2_HUMAN	Nuclear transport factor 2	Cytoplasmic	14478	5.10	7	50	1	A
53	P13804	ETFA_HUMAN	Electron transfer flavoprotein alpha-subunit	Mitochondrial matrix	35080	8.62	5	17	1	B
54	P14550	ALDX_HUMAN	Alcohol dehydrogenase [NADP+]	-	36573	6.32	14	47	1	A
55	P14618	KPY1_HUMAN	Pyruvate kinase, M1 isozyme	Cytoplasmic	57878	7.58	17	38	1	B
56	P14625	ENPL_HUMAN	Endoplasmin	ER lumen	92470	4.76	7	10	1	A
57	P15428	PGDH_HUMAN	15-hydroxyprostaglandin dehydrogenase [NAD(+)]	Cytoplasmic	28978	5.56	10	40	1	A
58	P15531	NDKA_HUMAN	Nucleoside diphosphate kinase A	Nuclear, Cytoplasmic	17149	5.38	10	53	1	A
59	P16475	MLEN_HUMAN	Myosin light chain alkali, non-muscle isoform	-	16799	4.56	7	45	4	A
60	P17661	DESM_HUMAN	Desmin	Cytoplasmic	53389	5.21	13	29	5	A
61	P17931	LEG3_HUMAN	Galectin-3	Nuclear	26057	8.61	7	24	1	B

Table 1. continued

No.	Accession Number	Entry name	Protein	Subcellular location	*Mr* (Da)	p*I*	Match	Seq. cov. (%)	Freq. (n)	Gel
62	P19404	NUHM_HUMAN	NADH-ubiquinone oxidoreductase 24 kDa subunit	Mitochondrial	22327	8.21	7	29	1	A
63	P19971	TYPH_HUMAN	Thymidine phosphorylase	-	49982	5.36	16	34	1	A
64	P21796	POR1_HUMAN	Voltage-dependent anion-selective channel protein 1	Mitochondrial, Plasma membrane	30642	8.63	6	34	1	B
65	P21964	COMT_HUMAN	Catechol O-methyltransferase, membrane-bound form	-	30037	5.26	7	37	1	A
66	P22626	ROA2_HUMAN	Heterogeneous nuclear ribonucleoproteins A2/B1	Nuclear	37430	8.97	8	23	2	B
67	P22695	UCR2_HUMAN	Ubiquinol-cytochrome C reductase complex core protein 2	Mitochondrial	48470	8.74	7	23	1	B
68	P23528	COF1_HUMAN	Cofilin, non-muscle isoform	Nuclear, Cytoplasmic	18503	8.22	6	35	1	B
69	P24572	MLES_HUMAN	Myosin light chain alkali, smooth-muscle isoform	-	16961	4.46	7	39	1	A
70	P24844	MLRN_HUMAN	Myosin regulatory light chain 2 smooth-muscle isoform	-	19739	5.09	8	43	2	A
71	P25325	THTM_HUMAN	3-mercaptopyruvate sulfurtransferase	Cytoplasmic	32966	6.00	8	29	1	A

Table 1. continued

No.	Accession Number	Entry name	Protein	Subcellular location	*Mr* (Da)	p*I*	Match	Seq. cov. (%)	Freq. (n)	Gel
72	P25705	ATPA_HUMAN	ATP synthase alpha chain, mitochondrial	Mitochondrial	59751	9.16	12	24	4	B
73	P25787	PSA2_HUMAN	Proteasome subunit alpha type 2	Cytoplasmic, Nuclear	25768	7.12	6	26	1	B
74	P27797	CRTC_HUMAN	Calreticulin	ER lumen	48142	4.29	10	25	1	A
75	P28838	AMPL_HUMAN	Cytosol aminopeptidase	Cytoplasmic	52772	6.29	7	18	1	B
76	P30040	ER29_HUMAN	Endoplasmic reticulum protein ERp29	ER lumen	8994	6.77	6	21	1	A
77	P30043	FLRE_HUMAN	Flavin reductase	Cytoplasmic	16995	6.58	11	72	1	B
78	P30044	AOPP_HUMAN	Putative peroxisomal antioxidant enzyme	Peroxisomal	21998	7.31	6	32	2	B
79	P30084	ECHM_HUMAN	Enoyl-CoA hydratase	Mitochondrial	31372	8.34	8	38	1	A
80	P30086	PEBP_HUMAN	Phosphatidylethanolamine-binding protein	Cytoplasmic	20926	7.43	9	54	2	B
81	P30085	KCY_HUMAN	UMP-CMP kinase	-	22223	5.44	7	46	1	A
82	P30101	PDA3_HUMAN	Protein disulfide isomerase A3	ER lumen	56783	5.99	13	28	3	A
83	P31930	UCR1_HUMAN	Ubiquinol-cytochrome C reductase complex core protein I	Mitochondrial	51629	5.96	10	20	1	A
84	P31948	IEFS_HUMAN	Stress-induced-phosphoprotein 1	-	62640	6.40	10	21	1	A
85	P32119	PDX2_HUMAN	Peroxiredoxin 2	Cytoplasmic	21892	5.66	13	41	3	A
86	P35232	PHB_HUMAN	Prohibitin	Cytoplasmic	29894	5.57	12	40	1	A
87	P37802	TAG2_HUMAN	Transgelin 2	-	22392	8.41	9	40	2	A

Table 1. continued

No.	Accession Number	Entry name	Protein	Subcellular location	*Mr* (Da)	p*I*	Match	Seq. cov. (%)	Freq. (n)	Gel
88	P40121	CAPG_HUMAN	Macrophage capping protein	Nuclear, Cytoplasmic	38518	5.88	7	20	2	A
89	P40926	MDHM_HUMAN	Malate dehydrogenase	Mitochondrial	35318	8.92	9	63	1	B
90	P45880	POR2_HUMAN	Voltage-dependent anion-selective channel protein 2	Mitochondrial	38093	6.32	6	26	1	B
91	P46777	RL5_HUMAN	60S ribosomal protein L5	Cytoplasmic	34448	9.76	11	31	1	B
92	P47985	UCRI_HUMAN	Ubiquinol-cytochrome C reductase iron-sulfur subunit	Mitochondrial	29652	8.55	5	16	1	B
93	P48637	GSHB_HUMAN	Glutathione synthetase	-	52385	5.67	11	22	1	A
94	P48735	IDHP_HUMAN	Isocitrate dehydrogenase [NADP]	Mitochondrial	53565	9.00	11	23	1	B
95	P49411	EFTU_HUMAN	Elongation factor Tu	Mitochondrial	49542	7.26	16	38	2	A
96	P50224	SUPM_HUMAN	Monoamine-sulfating phenol sulfotransferase	Cytoplasmic	34196	5.68	6	22	2	A
97	P51570	GAL1_HUMAN	Galactokinase	-	42273	6.04	13	36	1	A
98	P52565	GDIR_HUMAN	Rho GDP-dissociation inhibitor 1	Cytoplasmic	23207	5.02	6	28	1	A
99	P52566	GDIS_HUMAN	Rho GDP-dissociation inhibitor 2	Cytoplasmic	22988	5.10	6	23	1	A
100	P52742	Z135_HUMAN	Zinc finger protein 135	Nuclear	16350	6.80	7	34	1	B
101	P52907	CAZ1_HUMAN	F-actin capping protein alpha-1 subunit	-	32923	5.45	15	55	1	A
102	P55072	TERA_HUMAN	Transitional endoplasmic reticulum ATPase	Nuclear, Cytoplasmic	89323	5.14	15	27	2	A

Table 1. continued

No.	Accession Number	Entry name	Protein	Subcellular location	*Mr* (Da)	p*I*	Match	Seq. cov. (%)	Freq. (n)	Gel
103	Q01469	FABE_HUMAN	Fatty acid-binding protein, epidermal	Cytoplasmic	15165	6.60	9	60	1	A
104	Q01995	TAGL_HUMAN	Transgelin	Cytoplasmic	22480	8.88	15	61	4	A
105	Q03154	ACY1_HUMAN	Aminoacylase-1	Cytoplasmic	45885	5.77	8	20	1	A
106	Q04760	LGUL_HUMAN	Lactoylglutathione lyase	-	20720	5.24	7	33	2	A
107	Q05315	LPPL_HUMAN	Eosinophil lysophospholipase	-	16350	6.80	7	34	1	B
108	Q06830	PDX1_HUMAN	Peroxiredoxin 1	Cytoplasmic	22111	8.27	7	34	2	B
109	Q07244	ROK_HUMAN	Heterogeneous nuclear ribonucleoprotein K	Cytoplasmic, Nuclear	50977	5.39	8	20	3	A
110	Q13347	IF32_HUMAN	Eukaryotic translation initiation factor 3 subunit 2	Cytoplasmic	36502	5.38	5	18	1	A
111	Q15181	IPYR_HUMAN	Inorganic pyrophosphatase	Cytoplasmic	32660	5.54	12	43	1	A
112	Q15365	PCB1_HUMAN	Poly(rC)-binding protein 1	Nuclear	37526	6.66	9	33	1	B
113	Q16781	UBCC_HUMAN	Ubiquitin-conjugating enzyme E2-17 kDa	-	17138	6.13	8	48	1	A
114	Q16836	HCDH_HUMAN	Short chain 3-hydroxyacyl-CoA dehydrogenase,	Mitochondrial	34287	8.88	8	19	1	B
115	Q93079	H2BJ_HUMAN	Histone H2B.j	Nuclear	13892	10.31	8	41	1	B
116	Q99714	HCD2_HUMAN	3-hydroxyacyl-CoA dehydrogenase type II	-	26923	7.65	9	35	1	B
117	Q99798	ACON_HUMAN	Aconitate hydratase, mitochondrial	Mitochondrial	85659	7.14	12	24	2	B
118	1110572#		Type 1 iodothyronine deiodinase	-	2911	8.52	5	50	1	A

Table 1. continued

No.	Accession Number	Entry name	Protein	Subcellular location	*Mr* (Da)	p*I*	Match	Seq. cov. (%)	Freq. (n)	Gel
119	11275310#		Anti TNF-alpha antibody light-chain Fab fragment	Cytoplasmic	23395	6.91	7	48	2	A
120	11275326#		Anti HBs antibody light-chain Fab fragment	-	23133	6.48	5	31	1	B
121	1196417#		Deduced protein product shows significant homology to coactosin from Dictyostelium discoideum	-	15945	5.54	6	42	1	A
122	12654715#		Similar to glucose regulated protein, 58 kDa	-	36178	5.32	16	48	4	A
123	13630186#		Myosin regulatory light chain 2, smooth muscle isoform	-	19827	4.80	6	51	2	A
124	1710248#		Protein disulfide isomerase-related protein 5	-	46199	4.95	6	24	1	A
125	2144812#		Heat shock protein 27	-	22783	5.98	6	24	1	A
126	2773297#		RCL	-	19109	4.97	6	33	1	A
127	2460318#		RNA-binding protein regulatory subunit	-	19891	6.33	7	47	1	A
128	3337420#		SH3 domain binding glutamic acid-rich-like protein	-	12774	5.22	5	57	1	A
129	339685#		Transthyretin	-	12844	5.33	5	66	2	A
130	339958#		Skeletal muscle tropomyosin	-	26590	4.63	9	27	1	A
131	3779197#		Secreted cement gland protein XAG-2 homolog	-	19979	9.02	7	46	2	B
132	3893157#		Homology to a plant EST:RICS2753A	-	24858	5.63	6	29	1	A

Table 1. continued

No.	Accession Number	Entry name	Protein	Subcellular location	*Mr* (Da)	p*I*	Match	Seq. cov. (%)	Freq. (n)	Gel
133	4335941#		Leucine aminopeptidase	-	56650	7.58	8	16	1	A
134	4501889#		Actin, gamma 2 propeptide; actin, alpha-3	-	41877	5.31	7	23	2	A
135	5803011#		Enolase 2	-	47269	4.91	9	28	1	A
136	6470150#		BiP protein	-	70932	5.22	9	19	2	A

[a)]Accession numbers: SWISS-PROT database unless otherwise stated; #, GI number of NCBI database.
[b)]Entry name according to SWISS-PROT database.
[c)]Subcellular location is according to SWISS-PROT annotation; -, no information available or uncertain location.
[d)]Theoretical values.
[e)]Number of spots found on the two gels of Fig. 3A and 3B, that were identified to a given accession number.
[f)]The gel in Fig. 3 on which the protein was detected and identified.

Fig. 4 shows two examples (P15531 and P11021) of spot identification. We used two kinds of WWW search programs to identify proteins by peptide mass fingerprinting, MS-Fit of UCSF and ProFound of the Rockefeller University. The criteria used to accept identifications included the extent of sequence coverage, the number of peptides matched, the probabilistic score, and also whether human protein appeared as the top candidates in the first pass search where no restriction was applied to the species of origin. The results of identification are summarized in Table 1 and labeled on Fig. 3A and 3B.

Concluding remarks

In this study, a series of 2-DE maps have been established for soluble proteins of human stomach tissue. 243 protein spots corresponding to 136 different proteins of human stomach tissue have been identified and localized on two gel images. As far as we know, this is the first 2-DE maps to be reported for human stomach tissue proteins. They cover a pH range of 4-9 with two partially overlapping pH range gels of 4-7 and 6-9. Also, some spot identifications were mapped onto lower resolution gels of level 1 and 2, so that the 2-DE maps presented here are useful also for other investigators working with wide pH range gels. The 2-DE maps presented in this study are thought to be useful in future proteomics studies aimed at the identification of some potential markers and the understanding of the mechanisms involved in the pathologies of human stomach, such as chronic gastritis, gastroduodenal peptic ulcers, and gastric cancer.

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