Detection of biomarkers for lysosomal storage disorders using novel technologies
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Chapter Eight

Potential artefacts in proteome analysis of plasma of Gaucher patients due to protease abnormalities

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Abstract

The plasma proteome of type I Gaucher disease patients was investigated by 2D gel electrophoresis (2DGE). Using the classical procedure with 8 M urea treated plasma, several high molecular weight proteins were absent in Gaucher plasma specimens, while additional low molecular weight proteins were visible. The latter were identified as proteolytic degradation products. Adding small amounts of patient plasma to control plasma gave extensive protein breakdown. The presence of 2.2 M thiourea/7.7 M urea in the rehydration solution totally prevented breakdown. In the ‘urea only’ solution, protease(s) uniquely present in Gaucher plasma, appear to be still active towards other denatured plasma proteins at low pH. Therapy of patients results in gradual disappearance of proteolytic capacity in plasma specimens, indicating it to be related to presence of Gaucher storage cells. The proteolytic activity could be partly removed from Gaucher plasma samples by Concanavalin A, suggesting that glycoproteins are involved. Reduction of proteolysis by Pepstatin A and Leupeptin implies that cathepsins, proteases known to be overproduced by Gaucher storage cells, are involved. In conclusion, 2DGE Gaucher plasma proteomes should be interpreted cautiously given the abnormal high levels of proteases associated with this disorder.
Introduction

Type I Gaucher disease (GD) is the most common lysosomal storage disorder, characterized by accumulation of glucosylceramide in the lysosomes of macrophages as a result of a deficiency in the activity of glucocerebrosidase (EC 3.2.1.45) [1]. The deficiency results from mutations in the glucocerebrosidase gene. In the type I variant of Gaucher disease, massive lysosomal storage of glucosylceramide occurs exclusively in tissue macrophages, called Gaucher cells. These storage cells are mostly found in liver, spleen and bone marrow resulting in severe hepatosplenomegaly, pancytopenia and bone problems, such as avascular necrosis, pathological fractures, bone pain and bone crises [1].

Two successful therapies are now registered for type I Gaucher disease. Most commonly applied is enzyme replacement therapy, a treatment based on chronic intravenous administration of glucocerebrosidase [2,3]. Moderately to mildly affected patients also benefit from a different type of treatment, substrate reduction therapy. This therapy is based on oral administration of N-butyldeoxynojirimycin (Zavesca), an inhibitor of glucosylceramide biosynthesis [4].

The high costs associated with therapies for Gaucher disease have stimulated a search for plasma biomarkers that might help to establish cost-effective, individualized treatment regimens. Numerous abnormalities in plasma of Gaucher patients have been described (see Aerts et al. [5]). Two specific Gaucher cell markers have been identified: chitotriosidase and CCL18. Chitotriosidase (EC 3.2.1.14), a human analogue of chitinases from lower animals, shows a 1000-fold increased activity in plasma of symptomatic Gaucher patients [6]. Plasma chitotriosidase is now measured regularly for decision making regarding initiation and optimization of therapeutic interventions. Plasma CCL18 levels are elevated 10 to 50-fold in symptomatic Gaucher patients [7]. Measurement of plasma CCL18 is an alternative tool to monitor Gaucher patients [7-10] and is particularly useful for patients that are chitotriosidase deficient [11].

Although several clinically useful markers for disease progression have already been identified, additional plasma markers would further improve monitoring of Gaucher patients. A conventional technique in biomarker discovery is two dimensional gel electrophoresis (2DGE)(for reviews see Chambers et al. & Poon et al. [12,13]). With this technique, alterations in protein composition of biological samples can quickly be identified. We performed an analysis of the plasma proteome of type I Gaucher patients using 2DGE. Comparison of the plasma protein pattern of patients and normal subjects, in the pI range from 4 to 7, consistently showed surprisingly extensive differences. Protein patterns of patient plasma lacked several abundant proteins with a high molecular weight (e.g. albumin), while a new group of low molecular weight protein spots appeared. The basis for this striking phenomenon was studied and could be contributed to a pronounced increase in proteolytic capacity of Gaucher plasma specimens at conditions commonly employed during 2DGE for proteins in the acidic range. The results of these investigations are presented here.
Materials & methods

Gaucher disease patients and controls
All 5 patients with Gaucher disease type I studied were known by referral to the Academic Medical Center and received enzyme replacement therapy (algelucerase, imiglucerase, Genzyme, Cambridge, MA, USA) individualized dosing [14]. EDTA (ethylenediaminetetraacetic acid) plasma samples were obtained before and during therapeutic intervention. Control subjects consisted of 4 healthy volunteers. Approval was obtained from the Ethical Committee. Informed consent was provided according to the Declaration of Helsinki.

Sample preparation of plasma samples for 2DGE
An aliquot of 50 μL of human (Gaucher disease or control) EDTA plasma was directly mixed with 450 μL of rehydration solution. The rehydration solution consisted of 8 M Urea or 2.2 M Thiourea/7.7 M Urea (Sigma Chemical Company, St. Louis, MO, USA) with 2% (w/v) Triton X-100, 2% (v/v) of the appropriate IPG buffer (Amersham Pharmacia Biotech, AB, Sweden), 20 mM DTT (Sigma Chemical Company, St. Louis, MO, USA) and a trace bromophenol blue (BDH Chemicals Ltd, Poole, England). The sample was kept in rehydration solution at room temperature for at least 1 hour to obtain full denaturation and solubilization. To test the effect of protease inhibitors, they were added (when indicated) at the following concentrations: Leupeptin (ICN), Pepstatin A (ICN Biochemicals, Aurora, Ohio, USA), Chymostatin (ICN), Antipain (ICN) all at 0.1 mg/mL; PMSF (Sigma) at 1:100 (v/v) from a saturated solution in ethanol.

2DGE of plasma samples
The final diluted plasma sample was loaded on the first dimensional separation (350 μL for 18 cm IPG strips, pH 4-7 Linear gradient (L) and 150 μL for 7 cm IPG strips, pH 4-7 L). IPG strips are purchased from Amersham Pharmacia Biotech, AB, Sweden. Electrophoresis in the first dimension was run overnight using an 8-step program (1 minute at 200 V, 30 minutes at 200 V, 1 minute at 400 V, 30 minutes at 400 V, 1 minute at 600 V, 30 minutes at 600 V, 1.5 hr at 3500 V, and 21-48 hr 3500 V). Prior to loading the strip was incubated sequentially with 10 mg/mL DTT (Sigma) and 25 mg/mL iodoacetamide (Sigma)(both 30 minutes at RT). The second-dimension acrylamide gels were 10-18% and 1.0 mm thick (for 18 cm IPG strips) or 12% and 1.0 mm thick (for 7 cm IPG strips).

SDS-PAGE
Some EDTA plasma samples were also analyzed by SDS-PAGE only. For this purpose the samples were prepared for 2DGE as described above. Subsequently sample buffer was added and samples were heated for 3 minutes at 96°C.

Occasionally, sample buffer was not added and samples were not heated. In these cases SDS, glycerol, DTT and Tris were added separately to reach final concentrations comparable to those in sample buffer. The SDS-PAGE gels were 7, 12 or 16% and 1.0 mm thick.
Proteome analysis of Gaucher plasma using 2DGE

Protein staining, tryptic digestion and MALDI analysis

Protein spots selected for mass spectrometric analysis were cut from the gel after staining and destaining with Bio-Safe™ Coomassie Stain (Bio-Rad Laboratories, Inc., Hercules, CA, USA) or colloidal Coomassie stain (Sigma Chemical Company, St. Louis, MO, USA) according to the manufacturer or using silver nitrate. For MALDI analysis, protein-containing gel slices were digested with trypsin (Roche Molecular Biochemicals, sequencing grade) and extracted according to Shevchenko et al. [15]. Only peptides eluted with 20 mM NH₄HCO₃ were used in the analysis. After drying in a vacuum centrifuge, peptides were dissolved in 1% formic acid and 60% acetonitrile (6 μL). Eluted peptides were mixed 1:1 (v/v) with a solution containing 52 mM α-cyano-4-hydroxycinnamic acid in 49% ethanol/49% acetonitrile/2% TFA and 1 mM ammonium acetate or 52 mM α-cyano-4-hydroxycinnamic acid (Sigma-Aldrich Chemie BV) in 50% ethanol/50% acetonitrile, when the sodium/potassium ion contamination was low. Prior to dissolving, the α-cyano-4-hydroxycinnamic acid was washed briefly with chilled acetone. The mixture was spotted on a MALDI target plate and allowed to dry at room temperature. Reflector matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) spectra were acquired on a Micromass M@LDI (Wythenshawe, UK). The resulting peptide spectra were interpreted with Micromass proteinprobe software and analyzed with MASCOT peptide mass fingerprint software and databases (both available at http://www.matrixscience.com).

Analysis of proteolytic fragments obtained by incubation of model proteins with Gaucher plasma digestion

Recombinant chitinase (Genzyme), agalsidase beta (Fabrazyme, Genzyme) and purified bovine serum albumin (Sigma) were dissolved with PBS at respectively 1 mg/mL, 5 mg/mL and 7.5 mg/mL, and to 24 μL of each solution, 1 μL of human EDTA plasma of a Gaucher patient was added. Human EDTA plasma from healthy individuals was used as control. Aliquots (5 μL) of the resulting solutions were each 10-fold diluted with denaturing solution consisting of 8 M Urea with 0.4 % (w/v) DTT and incubated at RT for 90 minutes. Next, 1% (w/v) iodoacetamide (Sigma) was added (incubation for 45 minutes at RT). Aliquots of the resulting samples (10 μL) were 50-fold diluted with 0.1% TFA before being cleaned using 100 μL OMIX C18 tips (Varian inc., Palo Alto, CA, USA) as described by the manufacturer. The bound peptides were eluted with 0.1% TFA in 60% acetonitrile, mixed with matrix and spotted on a MALDI target plate as described above.

Peptide sequence analysis by oMALDI- or LC-MS/MS

MALDI-TOF MS/MS peptide sequencing was performed using a QSTAR-XL equipped with an oMALDI interface (Applied Biosystems/MDS Sciex, Toronto, Canada). For LC-MS/MS analysis, the peptide separation was accomplished using an Agilent 1100 series LC system, fitted with a true nanoscale reversed-phase HPLC setup involving Dean switching as described by Meiring et al. [16]. Peptide mixtures were 1:20 diluted with 0.1% TFA in water and 5 μL was injected onto a 2 cm 100 μm ID C18 trapping column.
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(Nanoseparations, Bilthoven, The Netherlands). After loading and washing for 5 minutes at a flow rate of 5 μL/min with 98% solvent A (0.1% formic acid in water) and 2% solvent B (0.08% formic acid in acetonitrile), the peptides were eluted onto a 37 cm 50 μm ID C18 analytical column (Nanoseparations) using a linear gradient of 5-40% B for 25 min, at a flow rate of 125 nL/min. The QSTAR-XL (Applied Biosystems/MDS Sciex) was also used for online electrospray ionization-mass spectrometry (ESI-MS) with a 20-10 μm ID uncoated Picotip (New Objective, Woburn, MA, USA) connected to the column via an in-house modified micro ionspray with liquid junction (operating around 4.7 kV). Survey scans were acquired from m/z 300–1,200 and precursors were selected for MS/MS from m/z 50–2,000 using automatic selection and dynamic exclusion scripts. Acquired peptide spectra were searched against a non-redundant protein sequence database (SwissProt/TREMBL) using the online MASCOT search engine.

Depletion of glycoproteins from plasma using Concanavalin A Sepharose 4B beads

For the depletion of glycoproteins, Con A Sepharose™ (Concanavalin A coupled to Sepharose 4B beads, GE Healthcare Bio-Sciences AB, Uppsala, Sweden) was used. Before use, pre-swollen Con A Sepharose beads were washed with PBS to remove preservative. Next, beads were taken up in PBS. An aliquot of 75 μL human (Gaucher disease or control) EDTA plasma was directly mixed with 100 μL bead solution. The samples were rotated at 4°C for 1 hour allowing optimal binding of glycoproteins. Samples were centrifuged for 10 minutes at 10600 × g at 4°C and 25 μL of supernatant was mixed with 225 μL of (8 M urea) rehydration solution and incubated for at least 1 hour to obtain full denaturation and solubilization. The final diluted plasma sample (150 μL) was loaded on 7 cm IPG strips pH 4-7 L strips (Amersham Pharmacia Biotech, AB, Sweden). Further electrophoresis was performed as described above.

Results

Comparison of 2DGE protein patterns of Gaucher patient and control plasma

2D comparisons of plasma of 4 different healthy individuals with plasma of 5 different Gaucher patients were performed. Both large 2D gels (18 cm IPG strips 4-7 L, SDS PAGE 10-18%) and small 2D gels (7 cm IPG strips 4-7 L, SDS PAGE 12%) showed striking differences. The 2D electrophoresis protein patterns found with plasma of 5 different Gaucher patients lacked several abundant high molecular weight proteins (Fig. 1A and 1B). Strikingly, a new group of low molecular weight protein spots could also be observed, which were absent in control plasma. Since the overall majority of the extra protein spots with a low molecular weight are in the same pI range as the protein spots with a high molecular weight that disappear, we hypothesized that these small proteins may be degradation products due to proteolytic activity in the Gaucher plasma. To test this, we analyzed several of the small protein products by in gel digestion with trypsin followed by MALDI mass spectrometry fingerprinting. They were identified as human serum albumin

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fragments, nicely coinciding with the observed disappearance of mature albumin from the top of the gel. Apart from albumin, α-1B-glycoprotein was broken down as deduced from disappearing spots. To extend this analysis we also looked at differing protein bands from 1D SDS PAGE gels loaded with samples prepared for analysis on a IEF 4-7 L strip. By careful analysis of disappearing protein spots and breakdown fragments with the aid of peptide mass fingerprinting we could identify several proteins that are broken down in Gaucher plasma under these conditions. They are listed in Table 1.

Figure 1. Comparison of 2D electrophoresis protein pattern of control plasma and Gaucher patient plasma. Silver-stained two-dimensional SDS-PAGE separation of EDTA plasma. (A) Control plasma denatured with 8 M urea. (B) Gaucher plasma denatured with 8 M urea. (C) Gaucher plasma denatured with 7.7 M urea/2.2 M thiourea. First dimension pH 4-7 IEF gradient (18 cm IPG strips), second dimension 10-18% gradient SDS PAGE. Molecular mass and pI are indicated to the left and above.
Table 1. Proteins broken down in Gaucher plasma upon denaturing with urea only.

<table>
<thead>
<tr>
<th>Name</th>
<th>Swiss-Prot ID</th>
</tr>
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<tr>
<td>Actin</td>
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<tr>
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<td>P01009</td>
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<td>α-2-HS-glycoprotein</td>
<td>P02765</td>
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<td>β-hemoglobin</td>
<td>P68871</td>
</tr>
<tr>
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<td>P01024</td>
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<tr>
<td>Ig heavy chain</td>
<td>Several</td>
</tr>
<tr>
<td>Ig light chain</td>
<td>Several</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>P02768</td>
</tr>
<tr>
<td>Transthyretin</td>
<td>P02766</td>
</tr>
</tbody>
</table>

Next we identified specific cleavage sites that had to be due to the protease(s) activated in Gaucher plasma. We used a combination of different peptide mass analyses (Tryptic in gel (1D and 2D) digestion followed by Peptide Mass Fingerprinting with MALDI-TOF and further digest analyses by oMALDI-MS/MS and LC-MS/MS). Of 53 proteolytic sites that could not be attributed to trypsin, the large majority (80%) was on the carboxyterminal side of a hydrophobic amino acid (14 x leucine, 8 x phenylalanine, 8 x valine, 5 x methionine, 5 x alanine, 1 cysteine and 1 isoleucine). The remainder was made up of: 3 x histidine, 3 x threonine, 2 x tyrosine and 1 aspartic acid, 1 asparagine and 1 tryptophane each. Obviously ‘authentic’ cleavage after K/R already present in the sample before in gel digestion can not be detected in this fashion. To handle this problem we incubated model proteins (chitotriosidase, agalsidase beta and bovine serum albumin, see materials and methods) with Gaucher plasma under ‘tryptic’ conditions (pH 7.4, 8 M Urea). This rendered only 7 proteolytic sites, lacking uniformity in carboxyterminal residues: 2 x glutamic acid, glutamine, leucine, serine, lysine and arginine. This heterogeneity suggests that various proteses are involved in the proteolytic modification.

Of note, although many high molecular weight proteins were apparently degraded in the Gaucher plasma specimens, some were resistant to breakdown (see Fig. 1). This indicates specificity in breakdown of proteins in Gaucher plasma specimens upon 2DGE.

Effect of thiourea on proteolytic activity in Gaucher plasma during sample preparation

Strikingly, the Gaucher related protein breakdown with 2DGE could be totally prevented by using a rehydration solution containing 2.2 M thiourea/7.7 M urea instead of 8 M urea only. This resulted in a protein pattern of Gaucher plasma which fully resembled the protein pattern of control plasma (Fig. 1). The presence of 2.2 M thiourea/7.7 M urea in the rehydration solution apparently inactivates proteases that in the ‘urea only’ rehydration solution are active and responsible for extensive protein breakdown.
Effect of mixing Gaucher plasma with control plasma

To test whether the protein breakdown in 2DGE analysis was due to the presence of an active protease or the absence of a specific inhibitor in Gaucher plasma, we performed mixing experiments. Gaucher plasma was added to control plasma (1:24) prior to sample preparation. Subsequently 2DGE was performed. The 2D gels showed that abundant proteins with a high molecular weight disappeared, while new spots with a low molecular weight appeared (data not shown). Thus, mixing Gaucher plasma with control plasma clearly demonstrated that breakdown was due to the presence of protease(s), and not to the absence of inhibitor(s), in Gaucher plasma.

Enzyme replacement therapy lessens proteolytic activity in Gaucher plasma during sample preparation

To investigate whether the capacity for protein breakdown as revealed 2DGE in plasma of patients was corrected during therapeutic intervention, we performed a 2D comparison of plasma of a Gaucher patient before and after successful enzyme replacement therapy. We used plasma samples of a symptomatic Gaucher patient, who displayed marked improvement of visceral and hematological problems. Therapeutic intervention resulted in normalization of the plasma protein pattern upon 2DGE (Fig. 2). Furthermore, a group of acidic proteins of about 37 kDa (probably a partial breakdown product) more pronounced in plasma of untreated Gaucher patients seems to diminish upon therapy (indicated with an
arrow in Fig. 2B). The comparison of plasma of a Gaucher patient before and after therapy was also made using only one-dimensional SDS-PAGE. The samples, prepared for 2DGE, were applied to a 7% and a 16% SDS-PAGE gel. The differences are less outspoken because all proteins instead of exclusively those with an acidic pI (4-7) are separated in one dimension only. Overall, however, the same conclusions could be drawn (results not shown). These findings stress the fact that the extensive protein breakdown in Gaucher plasma takes place during sample preparation in 8M urea solution prior to 2DGE and requires no isoelectric focusing.

Effect of preincubation with Con A Sepharose™ or protease inhibitors on proteolytic activity in Gaucher plasma

In an attempt to identify factors responsible for the extensive protein breakdown in Gaucher plasma with 2DGE, we employed Con A Sepharose™ (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) which recognizes glucosyl or mannosyl residues on proteins. Plasma of Gaucher patients was incubated with Con A Sepharose™ and supernatants were used for 2DGE. After the depletion with the immobilized lectin Concanavalin A, the excessive protein breakdown in 8M urea treated Gaucher samples was much less extensive (Fig. 3). This suggests that some glycoproteins with binding affinity for Con A Sepharose™ are involved in the protein breakdown in Gaucher plasma specimens. Next, we analyzed the effect of different protease inhibitors on the breakdown in our 2DGE assay (see Fig. 4 and materials & methods). We used Leupeptin (Fig. 4B), a combination of Chymostatin and Antipain (Fig. 4C), Pepstatin A (Fig. 4D), and PMSF (Fig. 4E). PMSF did not inhibit breakdown (compare Fig. 4A and E), Pepstatin A resulted in partial inhibition of breakdown, while both Leupeptin and the Chymostatin/Antipain combination totally inhibited breakdown under these conditions.

![Figure 3](image-url)

**Figure 3.** Effect of Con A Sepharose™ preincubation on proteolytic activity in Gaucher plasma during sample preparation. Coomassie-stained two-dimensional SDS-PAGE separation of EDTA plasma of a control and a Gaucher patient, with and without Con A depletion. Samples were denatured using 8 M urea. (A) Control plasma. (B) Gaucher plasma. (C) Gaucher plasma after preincubation with Con A Sepharose™. First dimension pH 4-7 IEF gradient (7 cm IPG strips), second dimension 12% SDS-PAGE.
Discussion

Our investigation on plasma protein patterns of Gaucher patients using conventional 2DGE in the acidic range led to an unexpected observation. Inspection of the gels indicated that in the case of patient plasma samples, common high molecular weight proteins were lacking and concomitantly novel low molecular weight proteins were present. The latter were shown to be degradation products. This extensive breakdown was not observed with IEF preincubations in the 3-10 NL range (so at neutral pH) implying the involvement of proteases with a preference for an acidic environment. The phenomenon was unique for samples of symptomatic Gaucher patients and was not observed in plasma specimens of normal subjects and was also almost absent in Gaucher patients successfully treated by enzyme replacement therapy. From this it can be concluded that unique factors involved in
protein breakdown are present in plasma of symptomatic Gaucher patients. Mixing experiments revealed that plasma samples from symptomatic Gaucher patients uniquely contain one (or several) protease(s) responsible for the noted protein breakdown with 2DGE rather than that some protease inhibitor is lacking.

We next noted that incubation of Gaucher plasma in 8 M urea containing solution was sufficient to cause the major protein breakdown earlier noted with 2DGE. This indicated that isoelectric focussing was not required for the phenomenon. Of particular note was the finding that treatment of Gaucher plasma samples with 2.2 M thiourea/7.7 M urea solution completely prevented the abnormal protein patter with 2DGE or 1DGE. The most likely explanation for our finding is as follows. Incubation of Gaucher plasma in 8 M urea solution results in denaturation of proteins, however specific proteases(s), elevated as the result of the presence of Gaucher storage cells in the patients, are not (fully) inactivated and able to degrade other denatured plasma proteins. An interesting alternative is that the protease(s) are actually activated in 8 M urea because of displacement of an inhibitory propeptide from the active site. In any case, incubation of Gaucher plasma in 2.2 M thiourea/7.7 M urea solution also causes inactivation of these Gaucher-related protease(s). This interpretation fits with the fact that the thiourea/urea condition has a stronger unfolding capacity than the ‘urea only’ condition [17].

The identity of the responsible protease(s) in Gaucher plasma is still unknown. Analysis of fragments of plasma and added model proteins released after incubation with Gaucher plasma in 8 M urea solution possibly can give some clue with regard to the identity of the protease(s) involved. Some preference for hydrophobic amino acids in the carboxyterminal position could be observed. This could be due to presence of an endoprotease with this specificity, but also to the presence of a (combination of) exopeptidases that stop at hydrophobic residues. Further investigation indicated that glycoproteins are likely to be involved, given the finding that Concanavalin A can at least partially remove the protease(s) activity. Both metalloproteinases (MMPs) and cathepsins are glycoproteins. Our experiments with inhibitors point to a key role for several of the cathepsins. Cathepsin B seems to be implicated as it can be inhibited by Antipain, Chymostatin and Leupeptin (Fig. 4). Cathepsin D is a candidate because of the partial inhibition by Pepstatin A. Cathepsin K and S are also inhibited by Leupeptin [18,19]. This finding fits nicely with the earlier observation by Moran and coworkers [20] that several cathepsins, including cathepsin D, K and S, are overproduced by Gaucher storage cells and their concentrations in plasma are increased in symptomatic patients.

It is very well conceivable that not a single, but rather a mixture of proteases, which are excessively present in plasma of symptomatic Gaucher patients, is responsible for the intriguing massive protein breakdown observed with 2DGE. Of note in this respect is the different composition of fragment termini we obtain upon incubation in 8 M urea at different pH (5.5 or 7.4). Complete elucidation of all responsible proteases will be very difficult to accomplish. It has to be realized that the observed phenomenon is a true artefact, being the result of the partial denaturing/activating effect of 8 M urea solution and the presence of unique proteases in Gaucher plasma. It is unequivocally known from
other types of analysis that the plasma of Gaucher patients does not show striking abnormalities in abundant high molecular weight proteins [21]. In conclusion, our study gave indirect indications for the abnormal presence of proteases in plasma of symptomatic Gaucher patients. Increases in concentrations of various cathepsins [20] and angiotensin-converting enzyme [5] in plasma of symptomatic Gaucher patients are already well known. Our investigation exemplifies that the results of 2DGE of plasma specimens should be interpreted with great care. In certain conditions under which specific proteases are elevated, like Gaucher disease, artificial results regarding the plasma proteome composition can always result from their unexpected activity.

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References

Chapter 8


