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Matrix-assisted laser desorption/ionization-quadrupole ion trap-time of flight mass spectrometry sequencing resolves structures of unidentified peptides obtained by in-gel tryptic digestion of haptoglobin derivatives from human plasma proteomes

Two-dimensional gel electrophoresis-separated and excised haptoglobin α2-chain protein spots were subjected to in-gel digestion with trypsin. Previously unassigned peptide ion signals observed in mass spectrometric fingerprinting experiments were sequenced using the matrix-assisted laser desorption/ionization-quadrupole ion trap-time of flight (MALDI-QIT-TOF) mass spectrometer and showed that the haptoglobin α-chain derivative under study was cleaved by trypsin unspecifically. Abundant cleavages occurred *C*-terminal to histidine residues at H23, H28, and H87. In addition, mild acidic hydrolysis leading to cleavage after aspartic acid residues at D13 was observed. The uninterpreted tandem mass spectrometry (MS/MS) spectrum of the peptide with ion signal at 2620.19 was submitted to database search and yielded the identification of the corresponding peptide sequence comprising amino acids (aa) aa65–87 from the haptoglobin α-chain protein. Also, the presence of a mixture of two tryptic peptides (mass to charge ratio *m/z* 1708.8; aa40–54, and aa99–113, respectively), that is caused by a tiny sequence variation between the two repeats in the haptoglobin α2-chain protein was resolved by MS/MS fragmentation using the MALDI-QIT-TOF mass spectrometer instrument. Advantageous features such as (i) easy parent ion creation, (ii) minimal sample consumption, and (iii) real collision induced dissociation conditions, were combined successfully to determine the amino acid sequences of the previously unassigned peptides. Hence, the novel mass spectrometric sequencing method applied here has proven effective for identification of distinct molecular protein structures.

Keywords: Haptoglobin / Mass spectrometric fragmentation / Matrix-assisted laser desorption/ionization-quadrupole ion trap-time of flight mass spectrometry / Peptide sequencing / Plasma / Protein structure characterization PRO 0381

1 Introduction

The characterization of protein patterns in tissues and body fluids of healthy individuals and of patients has the potential to serve as the basis for new diagnostic tools and the design of disease-specific therapies. However, as most proteins are present with several isoforms, detailed protein structure characterization becomes increasingly important in order to relate structure changes such as post-translational modifications with disease states. In addition, mass spectrometric peptide sequencing becomes an indispensible tool for the determination of disease-related sequence differences in proteins caused by protein processing events, alternative splicing, and/or single amino acid exchanges *e.g.* due to single nucleotide polymorphisms. Proteome analysis, *i.e.* two-dimensional gel electrophoresis (2-DE) [1, 2] followed by in-gel proteolytic digestion and mass spectrometric analysis of the peptide products [3–5] has become a powerful method for the separation and identification of proteins [6]. The necessities for concurrent detailed protein structure analyses led to the development of ESI-Q-TOF and, most recently, to MALDI-Q-TOF and MALDI-TOF/TOF tandem mass spectrometry methods, respectively, to perform MS/MS sequencing experiments [7–9] in order to pinpoint the structure specificities under study. For this purpose, many, if not all, peptide ion signals present in

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Abbreviations: aa, amino acid; **QIT**, quadrupole ion trap

 0173-0835/03/0606–851 $17.50+.50/0

a spectrum have to be fragmented and sequences and/or sequence modifications have to be elucidated by close inspection of the resulting MS/MS spectra. This is particularly true for "unidentified" peptide ion signals, as it has to become clear whether these ion signals carry structural information of the protein of interest, elucidating *e.g.* post-translational modifications. Alternatively, lack of identification of a peptide ion signal may be due to unknown contaminants that yield "unassigned" peptide ion signals.

Despite the success of the above-mentioned mass spectrometric fragmentation methods, limitation in their application is due to instrument design, that allows the performance of MS and/or MS/MS experiments only – not considering nozzle/skimmer fragmentation [10] that may be induced prior to MS/MS analysis in ESI mode. The use of ion traps, enabling the acquisition of MS^n spectra, helps to overcome these restrictions; and the latest developments of instruments combine these mass analyzers with MALDI ion sources [11–13]. Due to unforeseeable suppression effects and to differences in sample consumption *per* analysis between ESI and MALDI, it has been found advantageous to be able to fragment MALDI-derived peptide ions [14].

In this study, we applied a novel hybrid mass spectrometer comprising a MALDI ion source and a quadrupole ion trap (QIT), followed by a time of flight analyzer (MALDI-QIT-TOF mass spectrometer). This system combines the advantages of conventional MALDI-like rapid analysis time to achieve high-throughput with the ability to perform high efficiency ion trapping and MS/MS and MS^n analysis in the subfemtomole range [15]. We demonstrate the use of MALDI-QIT-TOF MS peptide fragmentation and sequencing for the analysis of unidentified peptides derived from haptoglobins that were produced by in-gel tryptic digestion after 2-DE of human plasma samples.

2 Materials and methods

2.1 Peptide sample preparation

2-DE gels were obtained from the 2-DE group of the Proteome Center Rostock. Gel pieces containing the proteins of interest were manually excised and subjected to in-gel digestion using an Investigator ProGest system (Genomic Solutions, Ann Arbor, MI, USA). Proteins were reduced, alkylated, and digested with trypsin as previously described [16], except that 15 μL of 25 mM NH_4HCO_3 containing 72 ng of sequencing grade trypsin (Promega, Madison, WI, USA) were used for digestion. After extraction of peptides from the gel, the samples were dried in a vacuum centrifuge. For peptide mapping, the peptides were resuspended in 5 μL of a solution that consisted of acetonitrile (60% v/v) mixed with aqueous 0.1% TFA (40% v/v). Of this peptide mixture – containing solution, 0.5 μL were spotted on a MALDI target plate simultaneously with 0.5 μL of matrix solution. The matrix solution was prepared such that a saturated solution of α-cyano-4-hydroxycinnamic acid (CHCA) dissolved in a mixture of acetonitrile (35% v/v) and aqueous 0.1% TFA (65% v/v) resulted. The same sample deposition method was used for placing samples on the target prior to mass spectrometric fragmentation and sequencing. However, 2,5-dihydroxy benzoic acid (DHB) solution with a concentration of 10 mg/mL was used as matrix in the latter case.

2.2 Mass spectrometric peptide mapping and database search

Peptide mixtures were analyzed by MALDI-TOF MS using a Reflex III mass spectrometer (Bruker Daltonik, Bremen, Germany), equipped with the SCOUT source and delayed extraction, and operated in positive ion reflector mode. The spectra were first calibrated externally [16] and then internally recalibrated using three peptides arising from trypsin autoproteolysis ($[M+H]^+$ 842.50, $[M+H]^+$ 2211.10, and $[M+H]^+$ 3323.77). Tryptic, monoisotopic peptide masses were searched against the Mass Spectrometry Protein Sequence Database (MSDB) using the Mascot software 1.8 (Matrix Science, London, UK) setting a mass tolerance of 50 ppm and two missed cleavage sites as fixed parameters. Spectra were analyzed using the BioTools software, V2.0 (Bruker Daltonik, Bremen, Germany).

2.3 Mass spectrometric peptide fragmentation and sequencing

Mass spectrometric peptide fragmentation and sequencing was performed on an Axima MALDI-QIT-TOF MS instrument (Kratos Analytical, Manchester, UK). This instrument (Fig. 1) employs a three-dimensional ion trap with a time of flight mass measurement stage [12]. MALDI is carried out utilizing a nitrogen pulsed laser (337 nm, 3–5 ns peak-width full width half maximum (FWHM). Ions are directed into the ion trap by means of an electrostatic lens assembly, designed to ensure high transmission for the MALDI beam into the ion trap and to minimize field strength directly above the sample surface.

The ion trap electrodes are all kept at static potentials during the ion introduction period, *i.e.* no trapping radio frequency (RF) is applied when the ions enter the vicinity of the ion trap. As ions fly through the introduction lens system they will separate along the ion optical axis according to their *m/z*, *i.e.* smaller ions will travel with higher veloci-

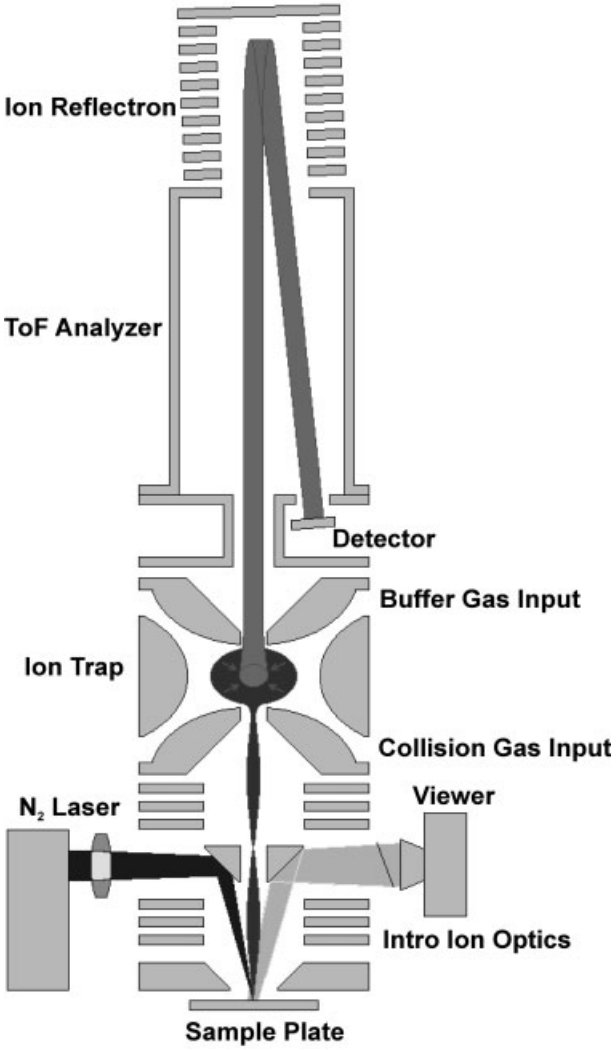

Figure 1. Schematic of the MALDI quadrupole ion trap-time of flight mass spectrometer.

ties than larger ones. While the smaller ions are stopped and reflected by the ion trap electrodes-induced field, increasingly larger ions are entering the trap region. The result of this process is to significantly broaden the range of masses found in the trap at any one time. At the point in time when the ions with the mass range of interest were found in the trap, appropriate electronics start up and maintain the required RF voltage to the ring electrode (rapid RF start-up technique). The combination of applying static voltages to the trap electrodes during introduction and rapidly transferring the system to trapping mode ensures very high trapping efficiency, theoretically approaching 100%.

The ion trap chamber is differentially pumped and supplied with two separate and independent gases. The continuous flow gas (helium) is used for collisional cooling of the desorbed ions. The pulsed gas (argon) is used to enhance ion cooling or to impose collisional induced fragmentation. Trapped ions relaxed into the centre of the ion trap from where they were selectively excited and ejected from the trap by means of appropriate waveforms, which were applied to the endcap electrodes (filtered noise field [17] waveforms). The ions that were selected to remain in the trap were excited with off-resonance sinusoidal waveforms, while at the same time collision gas (argon) was allowed to enter the trap. Collisional energy was adjusted by applying different amplitude waveforms. Fragment ions were cooled to the centre of the trap. The selection-excitation-collisional fragmentation cycle was repeated to induce multiple fragmentation MS^n.

Mass measurements were performed by extracting the cooled ions from the centre of the trap in the TOF analyzer. During the extraction sequence, the ring electrode was forced to ground, while the endcap electrode was pulsed to high voltages in order to create an extracting field along the trap axis. Exiting ions entered a floating flight tube with the same potential as the extraction endcap electrode (field-free region). TOF aberration due to energy spread was corrected by means of a double-stage gridless ion reflectron [18]. Ion arrivals were detected using a floating chevron multichannel plate detector. The signal was then collected by a 1 GHz transient recorder and accumulated by a digital signal processor (DSP) based control electronics before being transferred to the control computer for further processing and display by Launchpad software (Kratos Analytical Ltd., Manchester, UK). The spectra were first calibrated externally using a commercially available peptide calibration standard mixture with seven suitable calibrant compounds (Bruker Daltonik, Bremen, Germany). Afterwards they were internally recalibrated using three peptides from haptoglobin ($[M+H]^+$ 1267.66, $[M+H]^+$ 1590.80, $[M+H]^+$ 2033.00). MS/MS spectra were searched against the MSDB database using the Mascot software 1.8 (Matrix Science) setting a peptide tolerance of 80 ppm, a MS/MS tolerance of 0.8 Da and two missed cleavage sites as fixed parameters. Spectra were analyzed using Launchpad software (Kratos Analytical, Manchester, UK).

3 Results

3.1 2-DE analysis of human plasma proteins and mass fingerprint of haptoglobin α-chain allele-2 proteins

Our particular interest focussed on haptoglobin α-chain protein derivatives, as these form distinctly different migrating protein spots in 2-DE gels (Fig. 2A). The de-

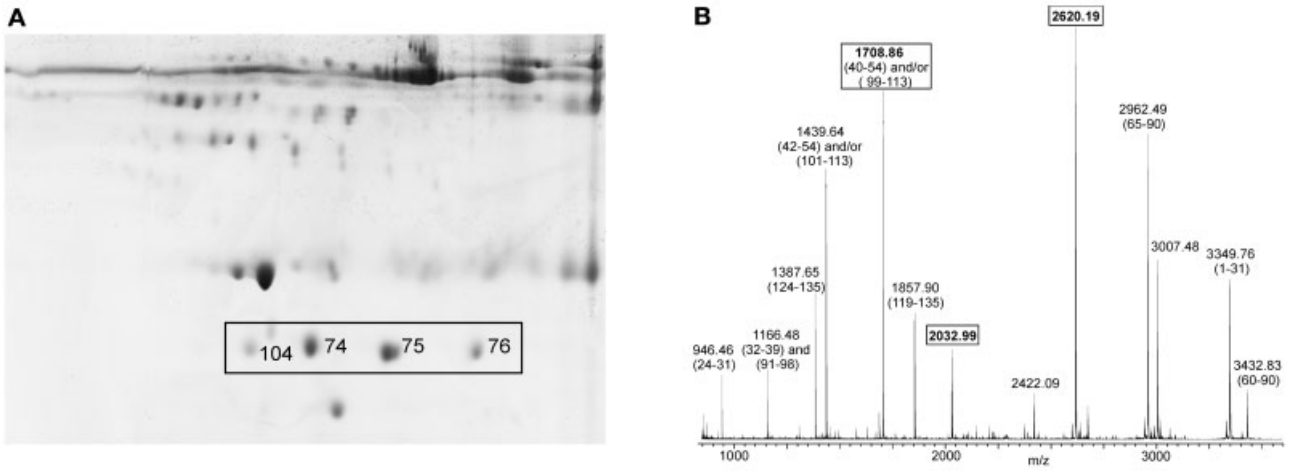

Figure 2. (A) 2-DE protein pattern of plasma proteins from a healthy blood donor. Proteins were mass spectrometrically identified [16]. The region containing the allele 2-derived haptoglobin spots (spots 74, 75, 76, and 104) is boxed. Total protein amount *ca.* 900 μg, pH range 4–7, colloidal Coomassie Blue staining. (B) MALDI-TOF mass spectrometric fingerprint obtained by in-gel tryptic digestion of haptoglobin α-chain derivative from spot 74. Numbers in the mass spectrum give precise *m/z* values for the detected peptide ion signals and automatically identified corresponding amino acid positions are indicated in parentheses. Boxed numbers indicate unidentified ion signals or ion signals that match two different sequence stretches that were subjected to mass spectrometric sequencing. CHCA was used as matrix.

picted protein pattern was obtained from one blood donor that was of particular interest, as it reproducibly showed rather unusual haptoglobin patterns when compared to those of patients and blood donors that were investigated earlier [16]. The automatically acquired MALDI mass fingerprints (*cf.* Fig. 2B) of the haptoglobin spots (spots 74, 75, and 76) identified all three spots as haptoglobin α-chain proteins, encoded by allele 2. Analyzing the intense ion signals in the spectra enabled automatic database search and identification, consistent with previous findings [16]. However, the mass spectra from the haptoglobin derivatives analyzed here were differing from the majority of previously investigated samples by presenting the most abundant, but not automatically assigned, ion signal at *m/z* 2620.19. In addition, some other intense ion signals (*e.g. m/z* 2032.99, 2422.09, 3007.48) remained unassigned in the mass fingerprints. As these ion signals potentially carry important structure information we investigated them further by mass spectrometric sequencing. The complete interpretation of the mass spectrum revealed that nearly 100% sequence coverage was obtained (Table 1). Only the *C*-terminal peptide (amino acid, aa136–142) was not observed in this spectrum.

By contrast, the ion signal found at *m/z* 1708.86 can be assigned to two sequence stretches of haptoglobin (aa40–54 and aa99–113; Fig. 3), that differ in mass by only 0.036 Da. This mass difference is not resolved in the

Table 1. MALDI-TOF MS assignments of peptides after tryptic in-gel digestion from haptoglobin[a)]

Sequence range	$[M + H]^+_{calculated}$	$[M + H]^+_{observed}$
1–23	2422.08	2422.09[b)]
1–28	3007.39	3007.48[b)]
1–31	3349.54	3349.76
14–31	2033.00	2032.99[c)]
24–31	946.47	946.46[b)]
32–39	1166.53	1166.48
40–53	1580.79	1580.77
40–54	1708.89	1708.86
40–59	2378.25	2378.20
42–54	1439.70	1439.64
42–59	2109.06	2108.99
60–90	3432.60	3432.83
65–87	2620.14	2620.19[b)]
65–90	2962.35	2962.49
73–90	2033.00	2032.99[c)]
91–98	1166.53	1166.48
99–113	1708.85	1708.85
99–118	2378.21	2378.20
101–113	1439.67	1439.64
101–118	2109.03	2108.99
119–135	1857.92	1857.90
124–135	1387.67	1387.65

a) Spot 74, see also Fig. 2
b) Unspecific tryptic proteolysis
c) Nontryptic proteolysis

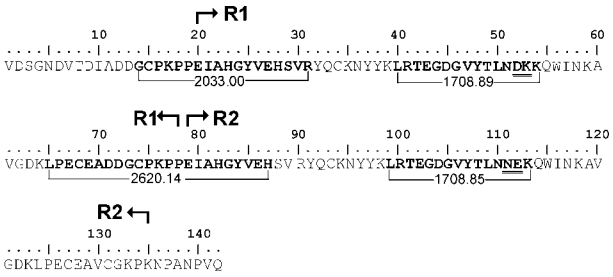

Figure 3. Amino acid sequence for the haptoglobin α-chain allele 2 protein according to database entry P00738 (SWISS-PROT). The amino acid sequence is depicted in the single letter code. The sequence stretches that were confirmed by mass spectrometric sequencing are printed in bold letters. Calculated peptide masses are given. The regions termed repeat 1 (R1) and repeat 2 (R2) are depicted. The distinctive sequence difference between the repeats is doubly underlined. Amino acid residue positions are given on top of the sequence.

MALDI-TOF mass spectrum. However, mass spectrometric sequencing enabled the identification of the two overlaying sequences (see below).

3.2 QIT-TOF MS sequencing of unassigned peptides from haptoglobin α-chain proteins

First we subjected the peptide producing the largest ion signal (*m/z* 2620.19) to QIT-TOF MS sequencing. The spectrum (Fig. 4A) showed intense fragment ion signals predominantly from the Y″-type ion series. Ions from the B-type ion series were also present (Table 2) and were joined by further ions derived from additional fragmentations such as loss of water and of ammonia. The uninterpreted MS/MS spectrum was submitted to database search and yielded the identification of the corresponding peptide sequence comprising amino acids 65–87 from the haptoglobin α-chain protein (Fig. 4B). This result confirmed the previous protein identification from the mass fingerprint data. In addition, it showed unambiguously that, for still unknown reasons, trypsin digestion caused cleavage after H87 of the haptoglobin α-chain protein derivative under study. Assuming that further cleavages after histidine residues may have occurred, we re-inspected the mass fingerprint spectrum (Fig. 2B) and assigned the ion signals at *m/z* 2422.09 and 3007.48 as cleavage products with cleavage sites after H23 and H28, respectively. It should be noted that the cleavage motif around H28 is identical to the cleavage site at H87.

Subsequently, we subjected the peptide with ion signal at *m/z* 2033.01 to QIT-TOF MS sequencing and the spectrum (Fig. 5) was interpreted to show fragment signals belonging to the haptoglobin sequence ranging from aa14 to aa31 and aa73 to aa90, respectively (Table 3). Again predominant fragment ion signals were obtained from the Y″-type ion series and the B-type ion series. Also accompanying signals due to loss of water and ammonia were found. Interestingly, the obtained sequence showed, that this peptide cleavage after D13

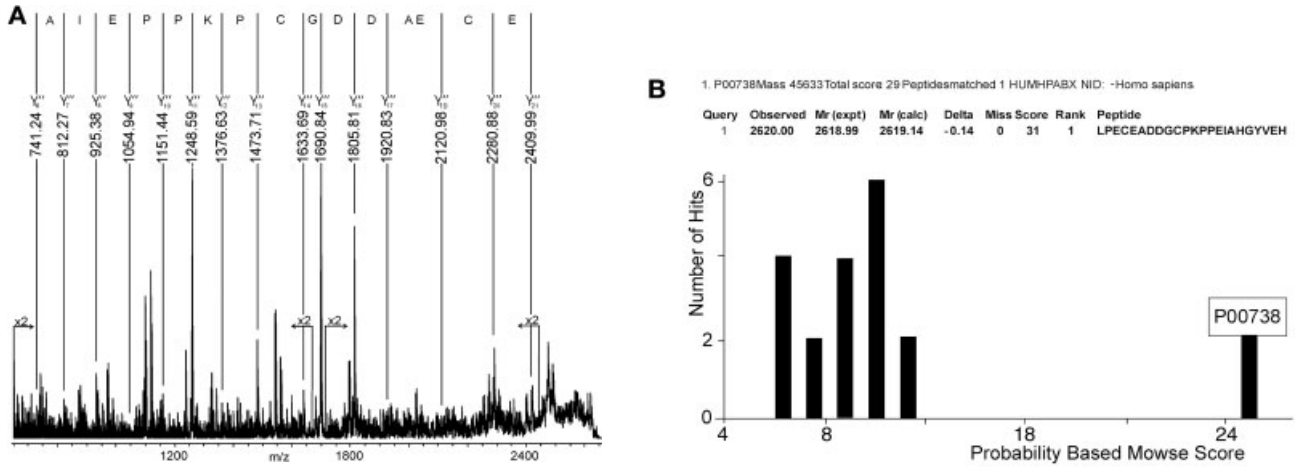

Figure 4. (A) QIT-TOF MS/MS analysis of peptide with ion signal at *m/z* 2620.09. The mass spectrometric fragment ions from the Y″-type ion series are indicated [30]. The determined partial amino acid sequence is depicted and was assigned to a haptoglobin peptide comprising aa 65–87. Magnification factor is given. DHB was used as matrix. (B) Database search result after submission of the uninterpreted MS/MS spectrum from (A). The search identified the sequence to belong to human haptoglobin (P00738) and resulted in an identification score of 31. The sequence was determined as an unspecific tryptic cleavage product that was *C*-terminally clipped after H87.

Table 2. MS/MS fragment masses for haptoglobin peptide with ion signal at *m/z* 2620.09[a)]

B series (calculated)	B series (observed)		Residue		Y″ series (observed)	Y″ series (calculated)
114.09	b)	1	Leu (L)	23		
211.14	b)	2	Pro (P)	22	b)	2507.06
340.19	b)	3	Glu (E)	21	2410.00	2410.01
500.22	b)	4	Cys (C)	20	2280.88	2280.96
629.26	b)	5	Glu (E)	19	2120.98	2120.93
700.30	b)	6	Ala (A)	18	b)	1991.89
815.32	b)	7	Asp (D)	17	1920.83	1920.85
930.35	930.34	8	Asp (D)	16	1805.81	1805.83
987.37	b)	9	Gly (G)	15	1690.84	1690.80
1147.40	b)	10	Cys (C)	14	1633.69	1633.78
1244.46	b)	11	Pro (P)	13	1473.71	1473.75
1372.55	b)	12	Lys (K)	12	1376.63	1376.70
1469.60	b)	13	Pro (P)	11	1248.59	1248.60
1566.66	b)	14	Pro (P)	10	1151.44	1151.55
1695.70	b)	15	Glu (E)	9	1054.94	1054.50
1808.78	b)	16	Ile (I)	8	925.38	925.45
1879.82	b)	17	Ala (A)	7	812.27	812.37
2016.88	2016.54	18	His (H)	6	741.24	741.33
2073.90	b)	19	Gly (G)	5	b)	604.27
2236.96	b)	20	Tyr (Y)	4	b)	547.25
2336.03	b)	21	Val (V)	3	b)	384.19
2465.07	2465.03	22	Glu (E)	2	b)	285.12
2602.13	2602.04	23	His (H)	1	b)	156.08

a) Spot 74; peptide aa65–87
b) Not observed

Table 3. MS/MS fragment masses for haptoglobin peptide with ion signal at *m/z* 2033.00[a)]

B series (calculated)	B series (observed)		Residue		Y″ series (observed)	Y″ series (calculated)
58.03	b)	1	Gly (G)	18		
218.06	b)	2	Cys (C)	17	b)	1975.98
315.11	b)	3	Pro (P)	16	1815.97	1815.95
443.21	b)	4	Lys (K)	15	b)	1718.90
540.26	b)	5	Pro (P)	14	1590.84	1590.80
637.31	b)	6	Pro (P)	13	1493.73	1493.75
766.36	b)	7	Glu (E)	12	b)	1396.70
879.44	879.41	8	Ile (I)	11	1267.65	1267.65
950.48	950.48	9	Ala (A)	10	1154.58	1154.57
1087.54	1087.54	10	His (H)	9	1083.57	1083.53
1144.56	1144.57	11	Gly (G)	8	946.45	946.47
1307.62	1307.61	12	Tyr (Y)	7	889.46	889.45
1406.69	1406.74	13	Val (V)	6	726.30	726.39
1535.73	1535.73	14	Glu (E)	5	626.98	627.32
1672.79	1672.79	15	His (H)	4	497.94	498.28
1759.82	1759.81	16	Ser (S)	3	b)	361.22
1858.89	1858.88	17	Val (V)	2	b)	274.19
2014.99	b)	18	Arg (R)	1	b)	175.12

a) Spot 74; peptide aa14–31 and 73–90, respectively
b) Not observed

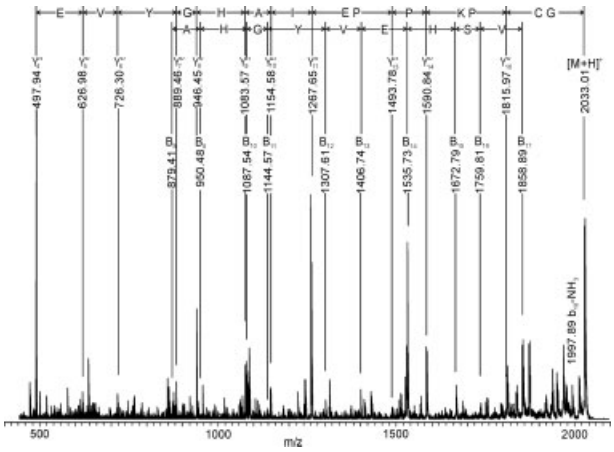

Figure 5. QIT-TOF MS/MS analysis of peptide with ion signal at *m/z* 2033.01. The mass spectrometric fragment ions from the Y″-type ion series and B-type ion series are indicated [30]. The determined partial amino acid sequence is depicted on top and was assigned to a haptoglobin peptide comprising aa14–31 and aa73–90, respectively. The sequence was determined as a cleavage product that was *N*-terminally clipped prior to G14 and G73, respectively. Magnification factor is given. DHB was used as matrix.

and D72, respectively, presumably caused by mild acidic hydrolysis that may have happened either during tryptic digestion (although this step was performed at pH 8.5) or subsequently during sample work-up. Mild acidic hydrolysis *C*-terminal to aspartic acid residues has been observed frequently [19] when multiple aspartic acid residues are present in a peptide as is the case for the *N*-terminal peptide of the haptoglobin α-chain protein (*cf.* Fig. 3).

QIT-TOF MS/MS analysis of peptides with ion signal at *m/z* 1708.56 also produced intense mass spectrometric fragment ions from the Y″-type ion series and B-type ion series, this time accompanied by some A-type ions (Fig. 6A, Table 4) [20]. The determined partial amino acid sequences are comprising aa40–54 and aa99–113 of the haptoglobin α2-chain protein, respectively. The sequences differ only by two amino acid exchanges that, however, result in nearly the same masses for the tryptic peptides (Fig. 3). QIT-TOF MS/MS/MS analysis of fragments with ion signal at *m/z* 1439.46 (*cf.* Fig. 6A) showed again the presence of the two overlaying sequences as can be seen by the enlargement around the B_{11} ion (Fig. 6B). The ion signals showed the expected mass difference of one dalton only in the B_{11} ions while for all other fragment ions the mass deviation between the ion signals was too small to be resolved. The relative intensities of the two B_{11} ions most likely mirror differing fragmentation yields for the corresponding partial sequences.

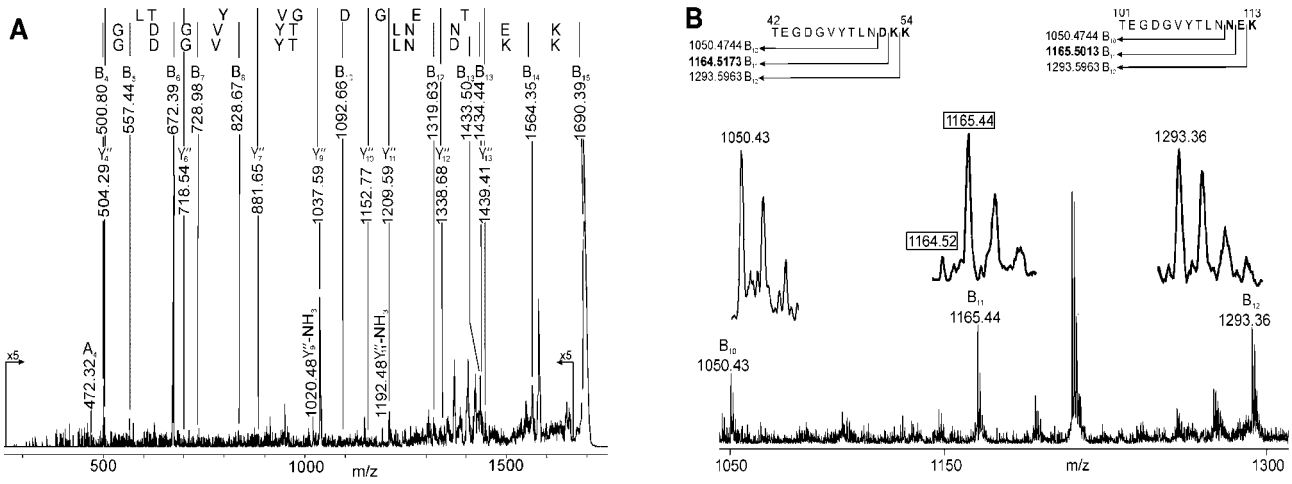

Figure 6. (A) QIT-TOF MS/MS analysis of peptides with ion signal at *m/z* 1708.56. The mass spectrometric fragment ions from the Y″-type ion series and B-type ion series and some A-type ions are indicated [30]. The determined partial amino acid sequences are depicted on top and were assigned to the haptoglobin peptides comprising aa40–54 and 99–113, respectively. The peptide mixture resulted in overlaying sequences. Magnification factor is given. DHB was used as matrix. (B) QIT-TOF MS/MS/MS analysis of fragments with ion signal at *m/z* 1439.46 (*cf* Fig. 6A). The spectrum shows the enlarged region around the B_{11} ion (*ca. m/z* 1050–1300). The mass spectrometric fragment ions from the B-type ion series are indicated [30]. The partial sequences are depicted on top and the theoretical masses of the B_{10}, B_{11}, and B_{12} ions are given. The peptide mixture resulted in overlaying sequences that showed a mass difference by one dalton only in the B_{11} ion, thus showing the presence of two peptide sequences. DHB was used as matrix.

Table 4. MS/MS fragment masses for haptoglobin peptides with ion signal at *m/z* 1708.56[a)]

B series[b)] (calculated)	B series (observed)		Residue		Y″ series (observed)	Y″ series[b)] (calculated)
114.1	c)	1	Leu (L)	15		
270.2	c)	2	Arg (R)	14	c)	1595.8
371.2	c)	3	Thr (T)	13	1439.13	1439.7
500.3	500.80	4	Glu (E)	12	1338.68	1338.6
557.3	557.44	5	Gly (G)	11	1209.59	1209.6
672.3	672.39	6	Asp (D)	10	1152.77	1152.6
729.3	728.98	7	Gly (G)	9	1037.59	1037.6
828.4	828.67	8	Val (V)	8	c)	980.5
991.5	c)	9	Tyr (Y)	7	881.65	881.5
1092.5	1092.66	10	Thr (T)	6	718.54	718.4
1205.6	c)	11	Leu (L)	5	c)	617.4
1319.7	1319.63	12	Asn (N)	4	504.29	504.3
1434.7	1434.81	13	Asü (D)	3	c)	390.2
1433.7	1433.50	13	Asp (N)	3		
1562.8	1562.35	14	Lys (K)	2	c)	
1562.8		14	Glu (E)	2		
1690.9	1690.39	15	Lys (K)	1	c)	147.1
1690.8						

a) Spot 75; peptide mixture: aa40–54 and aa99–113
b) Deviations in masses appear at the 2nd digit
c) Not observed

4 Discussion

Haptoglobin is an acute-phase protein and a transport protein that binds free hemoglobin which had escaped from destroyed erythrocytes and transports it for degradation into the reticuloendothelial system of the liver [21–23]. Native haptoglobin consists of α- and β-chains, which in man are encoded by a single gene [24–26]. Haptoglobin is presumably synthesized as a single precursor protein that is proteolytically processed after translation to form the α and β subunits [27–29]. Due to the occurrence of two common alleles, three major haptoglobin phenotypes exist. The β-chain is identical in all haptoglobin types. However, α-chains differ significantly in size. The homozygous haptoglobin 1-1 phenotype expresses the α1-chain of about 9 kDa, the homozygous haptoglobin 2-2 phenotype an α2-chain protein of about 16 kDa, whereas in heterozygous haptoglobin 2-1 both forms (α1- and α2-chains) are expressed [23]. 2-DE was carried out in order to separate the haptoglobin α-chain proteins and to study structure differences after in-gel digestion with trypsin. Mass spectrometric sequencing using the QIT-TOF MS instrument combines several positive features such as relatively simple sample preparation and easy performance of MS^n fragmentation and analysis for the characterization of peptide sequence specificities in the high attomole range [15].

5 Concluding remarks

The applied method has proven very efficient for the study of human proteomes and in general less sample-consuming than ESI-based mass spectrometric sequencing methods. Despite the fact that MALDI-QIT-TOF mass spectra show intense additional fragmentations, the acquired data were well suited for (i) uninterpreted database searches and (ii) *de novo* sequencing of proteins. Even tiny sequence variations were distinguishable. With this novel QIT-TOF MS sequencing technology, in all studied cases the parent ion signals were easily detected and the sequences were readily resolved. Two advantageous features, easy parent ion creation and real CID conditions, were combined to successfully assign amino acid sequences in unknown peptides.

We thank Ms. M. Sieb and Mr. M. Ulbrich for excellent technical assistance and the 2-DE group from the Proteome Center Rostock for providing 2-D gels. We acknowledge the Bundesministerium für Bildung und Forschung (BMBF) of the German Federal Republic for financial support (FKZ 01GG9831 and FKZ 03GL0042).

Received October 11, 2002

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