JOURNAL OF MASS SPECTROMETRY
J. Mass Spectrom. 2003; **38**: 277–282
Published online 24 January 2003 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jms.438

JMS

Analysis of post-translational modification and characterization of the domain structure of dynamin A from *Dictyostelium discoideum*

Andreas Schlosser,[1] Boris Klockow,[2] Dietmar J. Manstein[2] and Wolf D. Lehmann[1]*

[1] Central Spectroscopy, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany
[2] Department of Biophysics, Max Planck Institute for Medical Research, Jahnstrasse 29, D-69120 Heidelberg, Germany

Received 8 August 2002; Accepted 26 November 2002

The post-translational modifications of the 96 kDa protein dynamin A from *Dictyostelium discoideum* were analyzed using Q-TOF mass spectrometry. The accurate molecular mass of the intact protein revealed a covalent modification causing an additional mass of 42 Da. The modification could be identified as N-terminal acetylation by tandem mass spectrometry. Extracted ion chromatograms for the a_1 and b_1 ion of the tryptic T1 peptide were used to detect the acetylated peptide within 54 nanoelectrospray ionization tandem mass spectra. Owing to the accurate molecular mass of the intact protein, additional covalent modifications could be excluded. In addition to the covalent modification, the domain structure of dynamin A was determined by applying a combination of limited proteolysis, sodium dodecylsulfate polyacrylamide gel electrophoresis, automated tandem mass spectrometry and protein database searching.

KEYWORDS: dynamin A; limited proteolysis; domain structure; tandem mass spectrometry; acetylation

INTRODUCTION

Dynamins form a protein family within the GTP-binding protein superfamily. Their members share considerable sequence identity in the N-terminal region, whereas large variations in size and sequence are observed in the C-terminal part of these proteins. Dynamin A from *Dictyostelium discoideum* is composed of 853 amino acids and shows up to 44% sequence identity with dynamin-related proteins from other species. *DymA*$^-$ cells, which lack functional dynamin A, show alterations of mitochondrial, nuclear and endosomal morphology, a defect in fluid-phase uptake and an impairment in normal cytokinesis.[1] Dynamin A forms ring-like structures and helical assemblies in a nucleotide-dependent fashion supporting a mechanochemical action of dynamin A, in which tightening and stretching of a helix contribute to membrane fission.[2]

In the past few years, mass spectrometry (MS) has become the most powerful technique for the analysis of covalent protein modifications. Apart from isomerization and racemization, every covalent protein modification causes an alteration in molecule mass and thus is readily detectable with mass spectrometric techniques. Applying tandem mass spectrometry even isomerizations such as isoaspartate formation are detectable.[3] Low-energy collision-induced dissociation (CID) of modified peptides often causes a characteristic neutral loss of small molecules, e.g. loss of phosphoric acid from Ser/Thr-phosphorylated peptides[4] or loss of SO_3 from Tyr-sulfated peptides,[5] or yields specific fragment ions, e.g. the phosphotyrosine immonium ion at m/z 216.04,[6] sugar oxonium fragment ions from glycosylated peptides[7] or a fragment ion at m/z 104.02 derived from acrylamide-modified peptides.[8] These fragmentation reactions can be utilized for the specific detection of modified peptides in complex mixtures of protein digests. A drawback of this strategy is the risk of missing single modified peptides. The sequence coverage achieved by analysis on the peptide level in most cases is far from 100% owing to the loss of peptides during sample preparation. For example, hydrophobic peptides may be lost because of adsorption or precipitation and hydrophilic peptides may be lost during the desalting procedure owing to their low affinity to the C_{18} phase.[9] The so-called top-down approach introduced by McLafferty circumvents this fundamental problem by avoiding the cleavage of the protein.[10] The top-down characterization of a protein starts with the determination of the accurate molecular mass of the intact protein using Fourier transform ion cyclotron resonance (FTICR) mass spectrometry. Electron-capture dissociation (ECD) of the intact protein is then used to obtain sequence information including the localization of the covalent modifications.[11–13]

Here we describe a strategy for the characterization of covalent protein modifications using the accurate molecular

Abbreviations: CID, collision-induced dissociation; nanoESI, nanoelectrospray ionization; SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; HPLC, high-performance liquid chromatography; ECD, electron-capture dissociation; FTICR, Fourier transform ion cyclotron resonance; Q-TOF, quadrupole time-of-flight.

*Correspondence to: Wolf D. Lehmann, Central Spectroscopy, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany.
E-mail: wolf.lehmann@dkfz.de

mass of the intact protein and a combination of different protein cleavage methods. The post-translational modifications of the 96 kD protein dynamin A from *Dictyostelium discoideum* were determined with this strategy, since post-translational modifications might control the assembly of dynamin A into different ring and helical structures.[2] In addition, a straightforward strategy for the characterization of protein domain structure based on limited proteolysis, automated tandem mass spectrometry and protein database searching is described and applied to dynamin A. Knowledge of the domain structure of dynamin A is useful for the interpretation of the ring and helical structures observed by electron microscopy.[2]

EXPERIMENTAL

Protein cleavage and limited proteolysis

Dynamin A was purified from a dynamin A overexpressing cell line as described recently.[2] For analysis of the covalent modifications the protein was cleaved with BrCN, chymotrypsin and trypsin. The cleavage with BrCN was performed by dissolving about 10 pmol of dynamin A in 20 µl of 150 mM HCl, 150 mM BrCN, 8 M urea. This solution was incubated at room temperature overnight. The chymotrypsin digestion was carried out in 0.1 M Tris–HCl, 10 mM $CaCl_2$, pH 7.8 at 0 °C for 24 h with a chymotrypsin-to-protein ratio of 1 : 10 000 (w/w). The trypsin digestion was carried out in 20 mM NH_4HCO_3, pH 8.0 at 37 °C overnight with a trypsin-to-protein ratio of 1 : 100 (w/w).

Limited proteolysis experiments were performed with papain, subtilisin and trypsin with protease-to-protein ratios of about 1 : 10 000 (w/w) on ice. After different periods of times aliquots of the reaction solution were dissolved in sodium dodecylsulfate (SDS) buffer and separated by SDS polyacrylamide gel electrophoresis (SDS-PAGE). Bands were excised from the gel, cut into small pieces, destained with 40% acetonitrile, 0.1 M NH_4HCO_3, washed twice with water, dried under vacuum and rehydrated in 20 µl of trypsin solution (0.02 µg/µl trypsin in 20 mM NH_4HCO_3). Protein digestion was performed overnight at 37 °C. The supernatant was removed and the gel pieces were extracted twice with 10 µl of 50% acetonitrile. The extracts were combined with the supernatant, dried under a stream of nitrogen and redissolved in 20 µl of water for the desalting procedure.

Desalting

For determination of the accurate molecular mass of the intact protein, the protein solution was desalted using C_4-ZipTips (Millipore, Bedford, MA, USA). Equilibration of the C_4-ZipTips and washing of the bound protein were performed with 50% formic acid. For protein elution a solution of 50% formic acid and 50% 2-propanol was used. All peptide samples were desalted with C_{18}-ZipTips using 2% formic acid for equilibration and washing and 50% acetonitrile, 2% formic acid for peptide elution.

Mass spectrometry

Mass spectra were recorded on a Q-TOF2 quadrupole time-of-flight mass spectrometer (Micromass, Manchester, UK) equipped with a nanoelectrospray ionization (ESI) source. Spray capillaries were manufactured in-house using a type P-87 micropipette puller (Sutter Instruments, Novato, CA, USA) and coated with a semi-transparent film of gold in a type SCD 005 sputter coater (BAL-Tec, Balzers, Liechtenstein). The mass spectrometer was calibrated over the mass range 100–2000 using a 20 mM solution of phosphoric acid (in 30% 2-propanol). Tandem mass spectra were recorded in the automated MS to MS/MS switching mode with an m/z-dependent set of five collision offsets. For searching in the SwissProt protein database, the search engine Mascot was used.[14] For the assignment of the peptides generated by cleavage with BrCN and with chymotrypsin, the software tool Sherpa (www.hairyfatguy.com/Sherpa/Info.html) was utilized.

RESULTS AND DISCUSSION

Post-translational modification analysis

When analyzing the post-translational modifications of a protein, the accurate molecular mass of the intact protein is valuable information, since it is certain that all covalent modifications are present on the intact protein. In contrast, covalent modification analysis on the peptide level may be incomplete, particularly when only one type of protease is employed. In view of these facts, the first step in analyzing the post-translational modifications of dynamin A was molecular mass measurement of the intact protein. For this purpose the protein prepared from *Dictyostelium discoideum* was desalted using C_4-ZipTips. For a reliable determination of the accurate molecular mass of an intact protein, complete desalting is a crucial step, since salt adducts lead to incorrectly high experimental masses. To remove the non-covalently bound ions 50% formic acid turned out to be a suitable reagent, since owing to its good properties as a protein solvent the risk of protein precipitation is low. The peak width in the deconvoluted ESI spectrum is a good criterion to control successful desalting. If the experimental peak width is much greater than the calculated isotopic envelope, the molecular mass is probably incorrectly high. The deconvoluted nanoESI spectrum of intact dynamin A is shown in Fig. 1. Here the experimental peak width is 50 Da, about twice the calculated peak width, in our experience a tolerable value for a protein of that size analyzed with Q-TOF MS. The experimental average molecular mass is 96 148 Da, 42 Da higher than the molecular mass calculated from the amino acid sequence of the protein (SwissProt accession number Q94464).

A common post-translational protein modification that results in a mass shift of +42 Da is the acetylation of amino groups, especially of the N-terminal amino group. For verification and localization of the suspected acetylation, dynamin A was cleaved by different methods: cyanogen bromide cleavage and digestion by chymotrypsin and trypsin. The peptide mixtures of the cyanogen bromide cleavage and of the chymotrypsin digestion were analyzed with nanoESI-MS. The large peptides generated by these methods are readily assigned by accurate mass alone. In both cases a sequence coverage of 46% was obtained and large

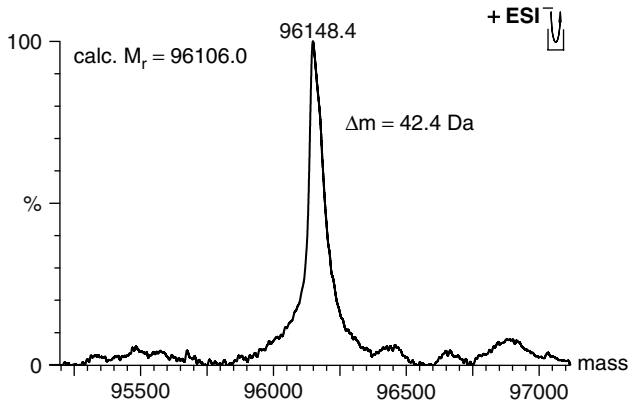


Figure 1. Deconvoluted nanoESI-TOF mass spectrum of dynamin A from *Dictyostelium discoideum*. The experimental molecular mass is 42 Da higher than the molecular mass calculated from the amino acid sequence.

parts of the protein were easily discovered as unmodified. A complete tryptic digestion of dynamin A was performed, resulting in small- and medium-sized peptides, which were characterized by nanoESI-MS/MS. The tandem mass spectra of the tryptic peptides were recorded in a data-dependent acquisition mode (automated MS to MS/MS switching).[15] The analysis of the tryptic digest resulted in a sequence coverage of 66%. In summary, the three alternative cleavages allowed us to assign 96% of the protein as unmodified.

To verify the suspected N-terminal acetylation of dynamin A, the tandem mass spectra of the tryptic peptides were systematically analyzed for specific fragment ions. If the genetically encoded N-terminal methionine is present, fragment ions at m/z 146.06 (a_1 ac-M) and m/z 174.06 (b_1 ac-M) are expected. If the N-terminal methionine is post-translationally removed, two fragment ions at m/z 130.05 (a_1 ac-D) and m/z 158.05 (b_1 ac-D) are expected. Owing to the acylation of the N-terminal amino group, the formation of a_1 and b_1 ions is feasible, which normally are not observed in the tandem mass spectra of peptides.[16] Applying automated MS to MS/MS switching to the tryptic digest of dynamin A, 54 peptide tandem mass spectra were recorded and stored in a single data file. This data file was analyzed for the presence of the acetylated a_1 and b_1 ions by generating extracted ion chromatograms for the above-mentioned m/z values. The result is shown in Fig. 2. The a_1–b_1 ion pair for ac-M is detected in two tandem mass spectra corresponding to doubly charged precursor ions at m/z 606.8 and 617.8, respectively. In contrast, the a_1–b_1 ion pair for ac-D is not observed in a tandem mass spectrum. To avoid false-positive signals in the extracted ion chromatogram the mass window was set to ±0.1 Da. The tandem mass spectrum of the doubly charged precursor ion at m/z 606.8 is shown in Fig. 3. The spectrum shows a complete y ion series from y_1 to y_9 which identifies the peptide ac-MDQLIPVINK, representing the acetylated N-terminal peptide of dynamin A. The tandem mass spectrum of the doubly charged precursor at m/z 617.8 identifies the sodium adduct of the same peptide. Hence the N-terminal amino acid of dynamin A is an acetylated methionine. This fits very well with the observed experimental molecular mass of the intact protein and thus allows the exclusion of other covalent modifications.

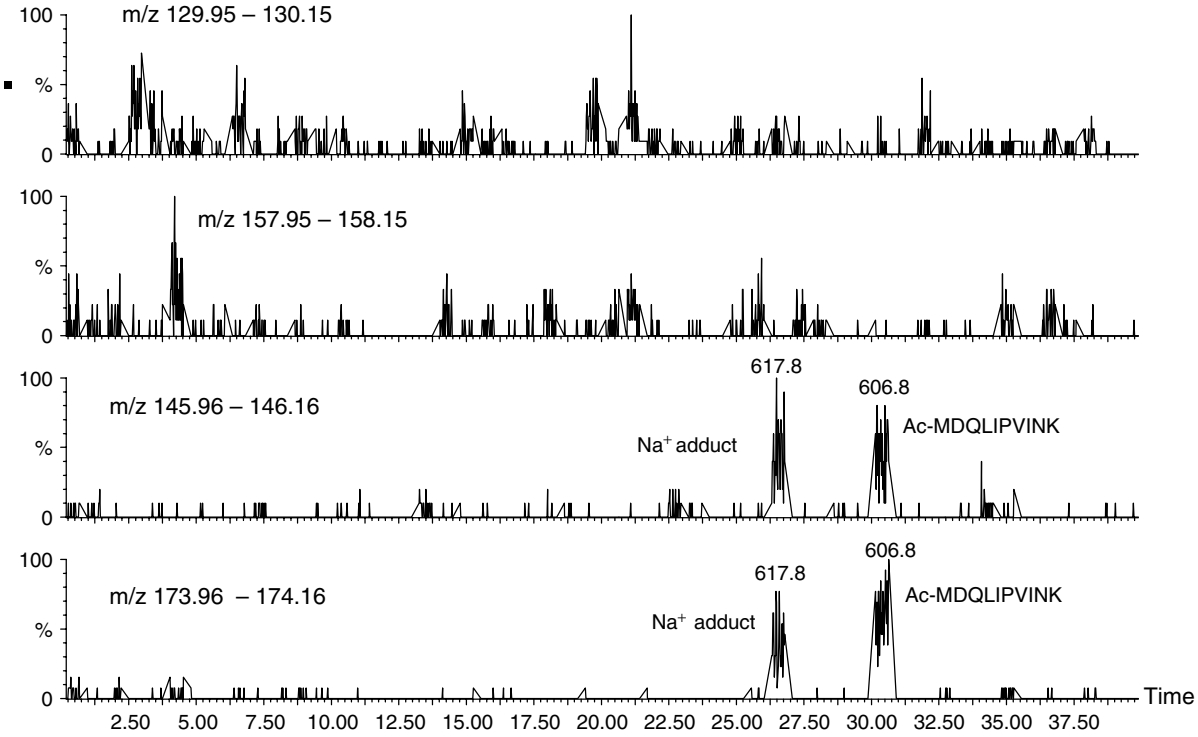


Figure 2. Extracted ion chromatograms for detection of the acetylated N-terminal tryptic peptide; 54 nanoESI tandem mass spectra were analyzed. Extracted ion chromatograms for (a) the a_1 ion for ac-D, (b) the b_1 ion for ac-D, (c) the a_1 ion for ac-M and (d) the b_1 ion for ac-M. The precursor ions at *m/z* 606.8 and 617.8 correspond to the acetylated N-terminal tryptic peptide of dynamin A and to its sodium adduct, respectively.

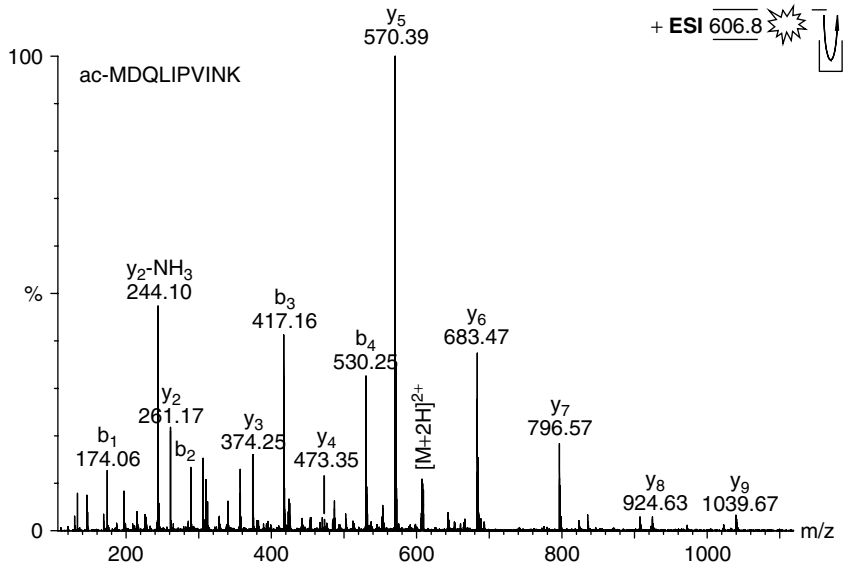


Figure 3. Tandem mass spectrum for the doubly charged acetylated N-terminal tryptic peptide derived from dynamin A. The spectrum shows a complete y ion series.

The described strategy for analyzing N-terminal acetylation using extracted ion chromatograms with accurate mass values is in principle also applicable for other N-terminal modifications, e.g. myristoylation, although myristoylated peptides are often very hydrophobic and therefore difficult to analyze. The corresponding a_1 and b_1 ions have m/z values of 240.23 and 268.23. In addition to these, the corresponding acylium ion (b_0) is observed at m/z 211.21.[17]

Domain structure

Functional protein domains are mostly compact folded structures, connected by linker regions. In general, these linker regions contain small hydrophilic amino acids, which built up a flexible stretch between the protein domains. Endoproteases cleave preferentially at the linker regions, since they are more easily accessible than the compact folded domains. Consequently, limited proteolysis can be used for the characterization of protein domain structure.[18] Mass spectrometry is often used as a tool for the characterization of the protein domains generated by limited proteolysis.[19–21]

To characterize the domain structure of dynamin A from *Dictyostelium discoideum*, limited proteolysis experiments with papain, subtilisin and trypsin were performed. The proteolytic reactions were stopped with SDS buffer after different periods of time, and the generated protein domains were separated by SDS-PAGE. Figure 4 shows as an example the SDS-PAGE analysis for the limited proteolysis experiment with trypsin. Three domains with an apparent molecular mass of 80, 60 and 14 kDa are obtained under the applied conditions. The corresponding Coomassie Brilliant Blue-stained bands were excised from the gel and the protein domains were digested in-gel with trypsin. The tryptic peptides were analyzed with nanoESI-MS/MS applying the automated MS to MS/MS switching mode. The combined and processed MS/MS data were used without manual interpretation for a search in the NCBI non-redundant protein database using the search engine Mascot.[14] The result of this search is summarized in Plate 1. Apart from keratin, dynamin A from *Dictyostelium discoideum* was the only significant hit in the database with a score of 513 (scores >43 indicate identity or extensive homology). Ten different peptides from the C-terminal region of the protein were identified, which together form a protein domain with an average molecular mass of 14 561.7 Da. This mass is in good agreement with the molecular mass of 14 kDa determined by SDS-PAGE.

The 60 kDa (T60) and the 80 kDa (T80) fragment of the limited trypsin proteolysis and the protein fragments generated by limited proteolysis with papain (P14, P20, P39 and P58) and subtilisin (S14 and S58) were analyzed in the same way. The results of these experiments are summarized in Fig. 5(a). The shaded gray areas represent regions identified with MS/MS data. The domain borders lie within the light-gray areas. The size of the light-gray areas represents the difference of the molecular mass determined

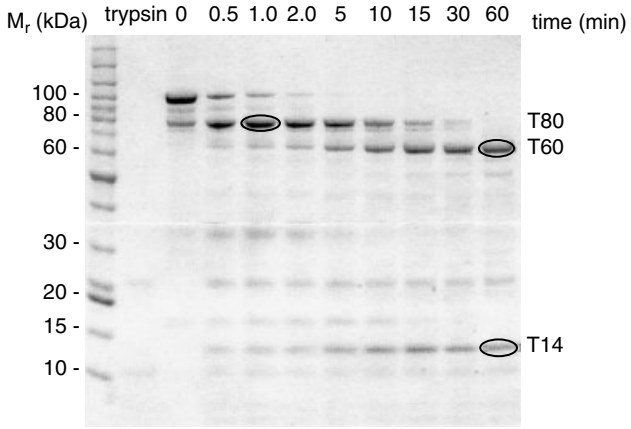


Figure 4. SDS-PAGE of the protein fragments generated by limited tryptic proteolysis of dynamin A. The marked gel bands (T14, T60 and T80) were excised and the protein domains were cleaved completely by trypsin and analyzed with nanoESI-MS/MS.

Mascot Search Results

Protein View

Match to: **gi|2689219**; Score: **513**
(X99669) dynamin like protein [Dictyostelium discoideum]
Found in search of D:\User\Schlosser\pkl-files\autopep_11.pkl

Nominal mass (M_r): **96047**; Calculated pI value: **6.71**
NCBI BLAST search of gi|2689219 against nr
Unformatted sequence string for pasting into other applications

Taxonomy: Dictyostelium discoideum

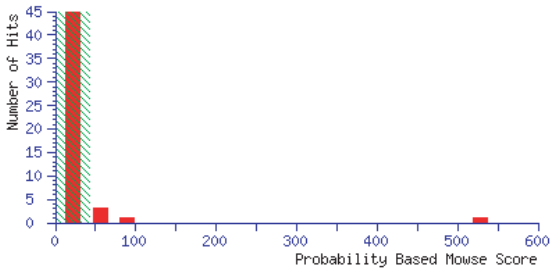
Cleavage by Trypsin: cuts C-term side of KR unless next residue
Sequence Coverage: **11%**

Matched peptides shown in **Bold Red**

```
    1 MDQLIPVINK LQDVFNTLGS DPLDLPQIVV VGSQSSGKSS VLENIVGRDF
   51 LPRGSGIVTR RPLILQLTHL PIADDGSQTQ EWGEFLHKPN DMFYDFSEIR
  101 EEIIRDTDRM TGKNKGISAQ PINLKIYSPH VVNLTLVDLP GITKVPVGDQ
  151 PTDIEQQIRR MVMAYIKKQN AIIVAVTPAN TDLANSDALQ LAKEVDPEGK
  201 RTIGVITKLD LMDKGTDAME VLTGRVIPLT LGFIGVINRS QEDIIAKKSI
  251 RESLKSEILY FKNHPIYKSI ANRSGTAYLS KTLNKLLMFH IRDTLPDLKV
  301 KVSKMLSDVQ GELSTYGDPL YDTKNSQGAL LLQIITIFSS NFKDAIDGKL
  351 TDLSNNELYG GARISYIFNE IYSHCVNNID PLEGISLNDI RTTMRNATGP
  401 RAALFIPEIS FELLVKKQVV RLEEPSAQCV EYVYDELQRI VSQLEAKELS
  451 RFINLKARVI EVVNNLLQKH KVPTKTMIEH LIKIETAFIN TSHPDFVGGE
  501 GIFESLYKKQ QLQQQNHLQQ LQDQYQQQQQ QQQQQQQQNG INNNQKGDNG
  551 NMNVNQQNMN QQNMNQQNQS TNPFLQQQQQ GQNKYPGGPP AQQQPNQQPN
  601 QLNKGPQNMP PNQSKPSSIP QNGPNNNNNN NNNNNRQDHQ QGSFFSSFFR
  651 ASPDPSLGQY GGANNSNNSN NPTSPINSSS NSGNNYNTFG GQQSSSSSSQ
  701 QLQQSSQSQY KTSYNNNNNS SSNNSSYNRY QDDFYGRGDK LNQVPSIIKA
  751 PDDLTSKEKF ETELIRELLI SYFNIVKKNV KDSVPKSIMH FLVNQSKEHI
  801 QNELVAALYK EELFDELLEE SPQISSKRKS CKAMIEILRK ANEIINEIRD
  851 FRN
```

Probability Based Mowse Score

Score is -10*Log(P), where P is the probability that the observed match is a random event.
Individual ions scores > 43 indicate identity or extensive homology (p<0.05).



Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Rank	Peptide
423.69	845.36	845.41	-0.06	0	44	1	APDDLTSK
506.27	1010.53	1010.61	-0.09	0	38	1	LNQVPSIIK
532.19	1062.37	1062.44	-0.07	0	40	1	YQDDFYGR
536.25	1070.49	1070.57	-0.08	0	60	1	ANEIINEIR
582.78	1163.54	1163.62	-0.08	1	37	1	EKFETELIR
600.30	1198.59	1198.67	-0.07	1	49	1	KANEIINEIR
652.30	1302.59	1302.68	-0.08	0	21	1	SIMHFLVNQSK
669.84	1337.66	1337.76	-0.10	0	65	1	ELLISYFNIVK
509.91	1526.69	1526.81	-0.11	0	(59)	1	EHIQNELVAALYK
764.36	1526.71	1526.81	-0.10	0	70	1	EHIQNELVAALYK
996.92	1991.83	1991.96	-0.13	0	105	1	EELFDELLEESPQISSK

Plate 1. Mascot search result for the T14 fragment. The T14 fragment was in-gel digested with trypsin and analyzed with automated MS/MS. The acquired data were used for a search in the NCBR non-redundant protein database without manual interpretation.

by SDS-PAGE (with a presumed error of ±10%) and the molecular mass calculated from the peptides identified with MS/MS. In Fig. 5(b), the domain structure of dynamin A as derived from the limited proteolysis experiments with papain, subtilisin and trypsin is shown. Four domains can be identified: domain I from position 1 to 180, domain II from position 180 to 508, domain III from position 508 to 730 and domain IV from 730 to 853. Figure 5(c) shows the domain structure of dynamin A as proposed from sequence comparison with other dynamin-like proteins.[1] The experimentally identified domains are very similar to the proposed domain structures. Domain IV correlates with the so called GTPase effector domain (GED) known from other dynamin-like proteins. Domain III correlates with a glutamine–asparagine–serine-rich domain (QNS), a domain that is not found within other dynamin-like proteins. Together domain I and II form a 58 kDa fragment. A fragment of the same size is generated by limited proteolysis of human dynamin 1,[22] a protein with extensive sequence homology to dynamin A in this region. The limited proteolysis with papain results in a 20 kDa N-terminal protein fragment, a fragment not observed with either trypsin or subtilisin. The cleavage site for this protein fragment is located around position 180 and lies within the GTPase domain. As is evident from the crystal structure of the GTPase domain of dynamin A (positions 2–316), the protein forms a loop structure in this region (positions 179–187).[23] This loop does not contain a trypsin cleavage site, but it seems to be sensitive for papain cleavage.

CONCLUSIONS

The covalent modifications of dynamin A from *Dictyostelium discoideum* have been determined by mass spectrometric analysis. Thereby, the accurate molecular mass of the intact protein was the key information. After the identification of the acetylated N-terminal tryptic peptide using extracted ion chromatograms, the molecular mass of the intact protein allowed us to exclude the presence of further covalent modifications. Extracted ion chromatograms turned out to be a useful tool for the detection of covalently modified peptides. The prerequisites for this strategy are, of course,

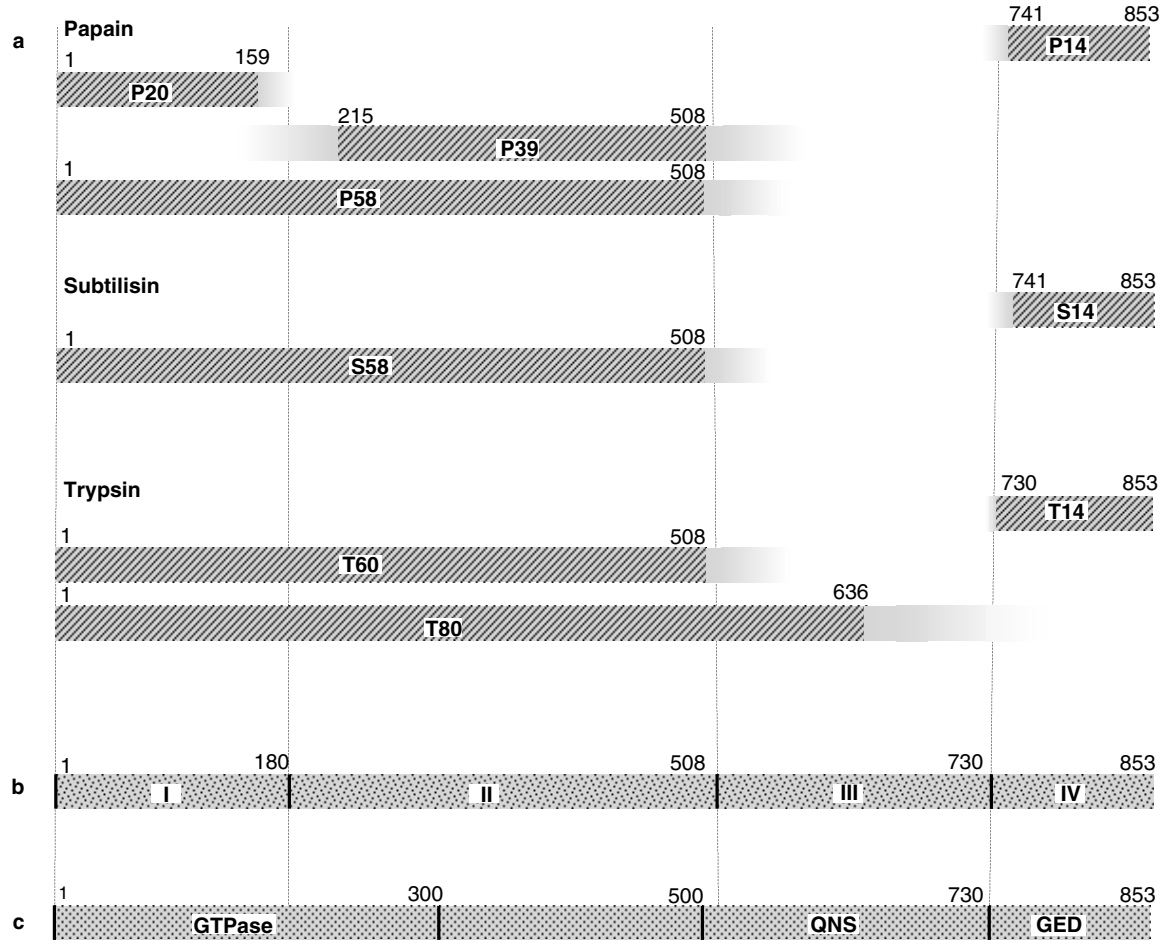


Figure 5. Domain structure of dynamin A from *Dictyostelium discoideum*. (a) Protein fragments generated by limited proteolysis of dynamin A with papain, subtilisin and trypsin. The shaded gray areas represent regions identified with MS/MS data. The domain borders lie within the light-gray areas. The size of the light-gray areas represents the difference of the molecular mass determined by SDS-PAGE (with a presumed error of ±10%) and the molecular mass calculated from the peptides identified with MS/MS. (b) Domain structure of dynamin A as derived from the data shown in (a). (c) Domain structure of dynamin A as derived from comparison with other dynamin-related proteins (after Wienke *et al.*[1]); QNS, glutamine–asparagine–serine rich domain; GED, GTPase effector domain.

that the modified peptide is present with a fairly high relative abundance and that the modified peptide yields a specific fragment ion upon CID. The molecular mass of the intact protein and the extracted ion chromatograms of specific fragment ions together are a valuable combination for the detection and localization of post-translational modifications.

Limited proteolysis, SDS-PAGE, automated MS/MS and protein database searching were used to characterize the domain structure of dynamin A. The benefit of this combination comes from the automated acquisition and interpretation of the MS/MS data. In the described approach, the borders of the analyzed domains can directly be revealed from the Mascot search result without manual interpretation of the MS/MS data. The domain structure of dynamin A obtained in this way is in good agreement with the predicted domain structure obtained by sequence comparison with other dynamin-like proteins, showing the applicability of the strategy.

REFERENCES

1. Wienke DC, Knetsch MLW, Neuhaus EM, Reedy MC, Manstein DJ. Disruption of a dynamin homologue affects endocytosis, organelle morphology, and cytokinesis in *Dictyostelium discoideum*. *Mol. Biol. Cell* 1999; **10**: 225.
2. Klockow B, Tichelaar W, Madden DR, Niemann HH, Akiba T, Hirose K, Manstein DJ. The dynamin A ring complex: molecular organization and nucleotide-dependent conformational changes. *EMBO J*. 2002; **21**: 240.
3. Lehmann WD, Schlosser A, Erben G, Pipkorn R, Bossemeyer D, Kinzel V. Analysis of isoaspartate in peptides by electrospray tandem mass spectrometry. *Protein Sci.* 2000; **9**: 2260.
4. Schlosser A, Pipkorn R, Bossemeyer D, Lehmann WD. Analysis of protein phosphorylation by combination of elastase digest and neutral loss tandem mass spectrometry. *Anal. Chem*. 2001; **73**: 170.
5. Rappsilber J, Steen H, Mann M. Labile sulfogroup allows differentiation of sulfotyrosine and phosphotyrosine in peptides. *J. Mass Spectrom*. 2001; **36**: 832.
6. Steen H, Küster B, Fernandez M, Pandey A, Mann M. Detection of tyrosine phosphorylated peptides by precursor ion scanning quadrupole TOF mass spectrometry in positive ion mode. *Anal. Chem*. 2001; **73**: 1440.
7. Huddleston MJ, Bean MF, Carr SA. Collisional fragmentation of glycopeptides by electrospray ionization LC MS and LC MS MS–Methods for selective detection of glycopeptides in protein digests. *Anal. Chem.* 1993; **65**: 877.
8. Jedrzejewski PT, Lehmann WD. Detection of modified peptides in enzymatic digests by capillary liquid chromatography/electrospray mass spectrometry and a programmable skimmer CID acquisition routine. *Anal. Chem.* 1997; **69**: 294.
9. Schlosser A, Bodem J, Bossemeyer D, Grummt I, Lehmann WD. Identification of protein phosphorylation sites by combination of elastase digestion, immobilized metal affinity chromatography, and quadrupole-time of flight tandem mass spectrometry. *Proteomics* 2002; **2**: 911.
10. Kelleher NL, Lin HY, Valaskovic GA, Aeserund DJ, Fridriksson EK, McLafferty FW. Top down versus bottom up protein characterization by tandem high-resolution mass spectrometry. *J. Am. Chem. Soc*. 1999; **121**: 806.
11. Sze SK, Ge Y, Oh H, McLafferty FW. Top-down mass spectrometry of a 29 kDa protein for characterization of any posttranslational modification to within one residue. *Proc. Natl. Acad. Sci. USA* 2002; **99**: 1774.
12. Ge Y, Lawhorn BG, ElNaggar M, Strauss E, Park JH, Begley TP, McLafferty FW. Top down characterization of larger proteins (45 kDa) by electron capture dissociation mass spectrometry. *J. Am. Chem. Soc*. 2002; **124**: 672.
13. Fridriksson EK, Beavil A, Holowka D, Gould HJ, Baird B, McLafferty FW. Heterogeneous glycosylation of immunoglobulin E constructs characterized by top-down high-resolution 2-D mass spectrometry. *Biochemistry* 2000; **39**: 3369.
14. Perkins DN, Pappin DJC, Creasy DM, Cottrell JS. Probability-based protein identification by searching sequence databases using mass spectrometry data. *Electrophoresis* 1999; **20**: 3551.
15. Hoyes E, Gaskell SJ. Automatic function switching and its usefulness in peptide and protein analysis using direct infusion microspray quadrupole time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom*. 2001; **15**: 1802.
16. Vaisar T, Urban J. Gas-phase fragmentation of protonated mono-*N*-methylated peptides. Analogy with solution-phase acid-catalyzed hydrolysis. *J. Mass Spectrom*. 1998; **33**: 505.
17. Eberle HB, Serrano RL, Füllekrug J, Schlosser A, Lehmann WD, Lottspeich F, Kaloyanova D, Wieland FT, Helms JB. Identification and characterization of a novel human plant pathogenesis-related protein that localizes to lipid-enriched microdomains in the golgi complex. *J. Cell Sci*. 2002; **115**: 827.
18. Taniuchi H, Anfinsen CB. An experimental approach to the study of the folding of staphylococcal nuclease. *J. Biol. Chem*. 1969; **244**: 3864.
19. Bantscheff M, Weiss V, Glocker MO. Probing the tertiary structure of multidomain proteins by limited proteolysis and mass spectrometry. *Eur. Mass Spectrom*. 1998; **4**: 279.
20. Bantscheff M, Weiss V, Glocker MO. Identification of linker regions and domain borders of the transcription activator protein NtrC from *Escherichia coli* by limited proteolysis, in-gel digestion, and mass spectrometry. *Biochemistry* 1999; **38**: 11 012.
21. Højrup P, Roepstorff P, Houen G. Human placental calreticulin: characterization of domain structure and post-translational modifications. *Eur. J. Biochem*. 2001; **268**: 2558.
22. Muhlberg AB, Schmid SL. Domain structure and function of dynamin probed by limited proteolysis. *Methods* 2000; **20**: 475.
23. Niemann HH, Knetsch MLW, Scherer A, Manstein DJ, Kull FJ. Crystal structure of a dynamin GTPase domain in both nucleotide-free and GDP-bound forms. *EMBO J*. 2001; **20**: 5813.