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Identification of Novel Site-Specific Alterations in the Modification Level of Myelin Basic Protein Isolated from Mouse Brain at Different Ages Using Capillary Electrophoresis–Mass Spectrometry

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Myelin basic protein (MBP) is a multifunctional protein involved in maintaining the stability and integrity of the myelin sheath by a variety of interactions with membranes and other proteins. MBP is subjected to extensive posttranslational modifications (PTMs) that are known to be crucial for the regulation of these interactions. Here, we report capillary electrophoresis–mass spectrometric (CE–MS) analysis for the separation and identification of MBP peptides that incorporate the same PTM at different sites, creating multiple localization variants, and the ability to analyze challenging modifications such as asparagine and glutamine deamidation, isomerization, and arginine citrullination. Moreover, we observed site-specific alterations in the modification level of MBP purified from brain of mice of different age. In total, we identified 40 modifications at 33 different sites, which include both previously reported and seven novel modifications. The identified modifications include Nα-terminal acetylation, mono- and dimethylation, phosphorylation, oxidation, deamidation, and citrullination. Notably, some new sites of arginine methylation overlap with the sites of citrullination. Our results highlight the need for sensitive and efficient techniques for a comprehensive analysis of PTMs.

1. Introduction

Myelin basic protein (MBP) is a major component of the myelin sheath that envelops the nerve axons of mammalian and submammalian species. It is responsible for the adhesion of the cytosolic surfaces of multilayered compact myelin and plays a crucial role in maintenance of the tight spiral wrappings of the myelin sheath required for proper functioning of nerves.[1] The MBP family arises from different transcription start sites of a larger gene complex called GOLLI (Genes of Oligodendrocyte Lineage), which has ten in humans and 11 exons in mice.[2] The so-called "classic" murine MBP isoforms arise from transcription start 3, the size isoforms range in nominal molecular mass from 14 to 21.5 kDa.

The sequence of MBP is highly conserved in higher animals and it is a very basic protein (pI = 11.87 for mouse MBP, 21.5 kDa isoform) caused by a large number of arginyl and lysyl residues. The protein undergoes a plethora of posttranslational modifications (PTMs) including phosphorylation, deamidation, methylation, and citrullination, resulting in a complex, diverse, and dynamic myelin basic proteome.[3]

Some of these PTMs like phosphorylation, deamidation, and citrullination reduce the net positive charge of MBP and affect the stability of the myelin sheath.[4] Citrullination is one of the most important and best characterized PTMs of MBP. Citrullination, also known as deimination, is an arginine-directed PTM that has the potential to alter the structure, function, and antigenicity of proteins. The conversion of arginine in the nonstandard amino acid citrulline is catalyzed by a family of calcium-dependent enzymes, called peptidylarginine deiminases.[5] At physiological pH, arginine has a positive charge due to the guanidinium group, whereas citrulline is neutral. Citrullination generates "altered-self" epitopes that may be antigenic, prompting autoimmune responses against previously benign proteins.[6] Altered calcium homeostasis accompanied by protein citrullination has been implicated in several age-related neurodegenerative disorders, including Alzheimer's disease,[7] rheumatoid arthritis,[8] and multiple sclerosis.[9]

Another key factor in the age-related denaturation of MBP is deamidation. Deamidation occurs on asparagine and glutamine residues and follows a rather complex mechanism, resulting not only in the introduction of a negative charge but also in a change in the primary structure, which in turn may affect secondary and tertiary protein structure. Glutamine deamidates via a glutarimide intermediate generating glutamate (α-glutamate) and isoglutamate (γ-gluamate).[10] Similarly, asparagine is converted to a mixture of aspartate and isoaspartate, typically in a 1:3 ratio.[11,12] Many factors can influence deamidation rates, such as protein

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Significance of the study

In this study, we used CE coupled to MS to separate and identify posttranslational modifications (PTMs) in myelin basic protein (MBP). This included label-free quantification of various modifications, including phosphorylation, deamidation, isomerization, mono- and dimethylation, and citrullination in brain of mice of different age. The excellent resolution obtained by CE enabled a clear assignment of deamidation and isomerization sites as well as the determination of isobaric deamidated and citrullinated peptides. We were able to identify seven new modifications in mammalian MBP. Of particular interest is the fact that several new sites of arginine methylation overlap with the sites of citrullination. Future studies are needed to establish the interplay between these PTMs of MBP in order to fully understand demyelination during aging and neurodegenerative diseases.

sequence, secondary structure, pH, temperature, ionic strength, and other solution properties. In general, half-lives of asparagine deamidation at neutral pH and physiological temperature range from 0.5 to 500 days, while those of glutamine range from about 100 to 5000 days.[13] Moreover, aspartate and α-glutamate can also be directly isomerized into isoaspartate and γ-glutamate, respectively. The succinimide formation from aspartate is ~10–40 times slower than from asparagine at neutral pH.[14] Isomerization of aspartate and glutamate may be especially disruptive because a "kink" in the protein conformation is generated, potentially leading to altered functions of the protein. Formation of isomerization products plays a diverse and crucial role in aging, cancer, neurodegeneration, and other diseases.[15–17]

The analysis of modifications such as asparagine and glutamine deamidation, aspartate and glutamate isomerization, and arginine citrullination is very challenging. A reliable separation of such modified peptides is crucial for unambiguous distinction by mass spectrometry. Deamidation and citrullination add only 0.984 Da to the mass of the intact molecule. When the modified peptides coelute, the m/z signal will fall within the isotopic cluster of the unmodified peptide, which hinders a successful identification of the PTM in conventional bottom-up proteomics. Analytical techniques for the identification and quantification of isomerized products are even more challenging primarily because they have identical mass and formal charge. Furthermore, mass spectrometric analysis of MBP is technically complicated due to the excess of basic residues, the large number of PTMs, and the very low level of some of these modifications in their natural states.

Given the pivotal role of PTMs in the regulation of cellular environment, there is a constant effort to develop novel, highly sensitive, and sophisticated PTM identification techniques. A promising technology is "CE–MS", a combination of electrophoresis and MS. Our group and others recently demonstrated that CE–MS can be used effectively to study posttranslationally modified peptides and proteins.[18–24] In the present study we systematically investigated the separation and differentiation of multiply modified MBP peptides using CE–MS. Myelin was purified from brain of mice of different age to identify age-dependent alterations in the modification level of MBP.

2. Experimental Section

2.1. Materials

Trypsin (mass spectrometry grade) and endoproteinase Lys-C (rLys-C) were obtained from Promega (Mannheim, Germany); 1,4 dithiothreitol was purchased from Biomol (Hamburg, Germany); iodoacetamide from GE Healthcare (Vienna, Austria); Protease inhibitor Complete-EDTA free from Roche (Mannheim, Germany); Rapigest from Waters (Vienna, Austria). Chymotrypsin (sequencing grade), and all other chemicals were purchased from Sigma–Aldrich (Vienna, Austria). Water was purified with a Millipore Milli-Q Academic water purification system (Vienna, Austria).

2.2. Sample Preparation

Myelin was purified from mouse brain by a method described by Jahn et al.[25] The tissues were homogenized using a Dounce homogenizer followed by an Ika Turran T8. Myelin can be considerably enriched from the homogenized nervous tissue by sucrose gradient centrifugation as the low-density membranous material accumulates at the interphase between 0.32 and 0.85 M sucrose. All solutions contained a protease inhibitor. The myelin pellet was dissolved in 250 μl 50 mM NH_4HCO_3 pH 8.0 containing 0.2% Rapigest.

For chymotryptic digest 25 μl of the myelin fraction was reduced with 25 μl dithiothreitol (50 mM) at 56°C for 30 min, digested at room temperature for 4 h adding 0.3 μg chymotrypsin, and alkylated with 25 μl iodoacetamide (200 mM) at room temperature for 20 min. Same protein amount and DTT was used for enzymatic digestion with trypsin or Lys-C, 1 μg of each enzyme was added, and digestion took place at 37°C for 6 h (trypsin) and 16 h (Lys-C), respectively, followed by alkylation. Resulting peptides were desalted using PerfectPure C-18 pipet tips (Eppendorf, Austria). All samples were lyophilized, resolubilized in 50 mM ammonium acetate buffer (pH 4.0), and stored at –20°C until analysis.

2.3. Capillary Electrophoresis–Mass Spectrometry (CE–MS)

For CE–MS analysis a CESI 8000 (integration of capillary electrophoresis and electrospray ionization into a single device , Sciex, Brea, CA) equipped with a neutrally-coated fused-silica capillary with a porous tip acting as nanospray emitter (Sciex, Brea, CA) (total length: 90 cm, id: 30 μm, od: 150 μm) was coupled via an ESI module to a Thermo Scientific Q Exactive HF (Bremen, Germany). Prior to each analysis the system was rinsed with background electrolyte (BGE, 0.1 M acetic acid) for 3 min at 50 psi. The sample was injected by applying a pressure of 5 psi for 20 s (17 nL) followed by an injection plug of BGE (5 psi for 5 s). The separation was performed at +30 kV with pressure applied

at the capillary inlet. The pressure profile was: 0–10 min, 0.5 psi; 10–40 min, 1.0 psi; from 40 min, 3 psi. For each enzymatic digest three technical replicates were performed.

2.4. Mass Spectrometry Data Acquisition

The Q Exactive HF mass spectrometer was operating in data dependent mode to switch between MS and MS/MS acquisition. Survey full scan MS spectra were acquired with a resolution of R = 60 000 at an automatic gain control (AGC) target of 3e6 in profile mode. To generate MS/MS spectra the ten highest precursors were selected for higher energy collision dissociation applying a normalized collision energy of 28.0. Fragments were scanned with a resolution of R = 30 000 at an AGC target of 5e5. Dynamic exclusion was set to 7 s. Unassigned and singly charged peptides were excluded from higher energy collision dissociation fragmentation.

2.5. Data Analysis and Protein Identification

Proteome Discoverer version 2.1.1.21 (ThermoScientific) with search engine SEQUEST HT was used for data analysis. Raw data obtained by CE–MS were searched against the UniProt mouse database (16 732 entries), to which 13 sequences of the mouse MBP isoforms were added. The following settings were applied: Depending on the enzyme used different missed cleavages were allowed; chymotrypsin, 5; trypsin, 4; endoproteinase Lys-C, 3; fixed modification was carbamidomethylcysteine; variable modifications were oxidation of methionine; deamidation of asparagine, glutamine, and arginine; phosphorylation of serine, threonine and tyrosine; mono- and dimethylation of arginine; N-terminal acetylation; and loss of methionine. Precursor mass tolerance was set to 10 ppm. The fragment mass tolerance was 20 mmu. False discovery rate for proteins and peptides was set to 1%.

3. Results

3.1. CE–MS Analysis of Myelin Basic Protein (MBP)

For an in-depth analysis of MBP, myelin was purified from whole brain of mice aged 13 weeks using sucrose density gradient fractionation (see Experimental Section). This procedure allows the enrichment of the classical myelin proteins, such as 2,3-cyclic-nucleotide 3-phosphodiesterase, myelin associated glycoprotein, MBP, myelin OL glycoprotein and myelin proteolipid protein, which are known to constitute more than 90% of total myelin proteins in the rodent.[26]

Triplicate CE–MS analysis of the chymotryptic digest of whole myelin yielded 76 different proteins including all classical myelin proteins (Supporting Information Table 1). Three isoforms of murine MBP appeared to be present in the preparation, which differ in their molecular weights due to alternative RNA splicing of a primary transcript of the MBP structural gene. The approach yielded 99% sequence coverage of the full-length 21.4-kDa classic MBP isoform 4 with 32 modifications at 25 different sites (Supporting Information Table 2). The identified modifications included Nα-terminal acetylation, mono- and dimethylation, phosphorylation, oxidation, deamidation, and citrullination. Most of the modified peptides were present in very low amounts. Therefore, we were interested to examine possible alterations of the MBP modification status in older mice, as levels of MBP deamidation and citrullination are known to increase in aged humans and multiple sclerosis patients.[27]

3.2. Age-Dependent Variation of the MBP Modification Status

For this purpose, myelin purified from whole brain of mice aged 53 weeks was investigated by CE–MS. Triplicate analysis yielded 74 different protein groups and 98% sequence coverage of MBP isoform 4 with 31 modifications at 25 different sites (Supporting Information Table 3 and 4). For identification of the isomerization products isoaspartate and isoglutamate, we followed our recently published work where we showed that CE separates aspartate and isoaspartate consistently with the same migration order and selectivity.[20] The detection of the isomerized peptide forms at two different migration times, with a difference of at least 1 min between each of them, enables the determination of the two forms. Following this approach, five isomerized aspartate peptides could be identified. In both young and adult mice the same MBP modifications were obtained, albeit in different amounts. Due to highly reproducible migration times and MS/MS fragmentation patterns an unambiguous assignment of even very low abundant modified peptides was possible.

Figure 1 shows the extracted ions of MBP (13LATASTM**D***HA**R***H24) from adult mice, which was found to be isomerized on D20 and citrullinated on R23. The isomerized peptide could be assigned because of the shift in migration time from aspartate to isoaspartate. The calculated separation selectivity $\alpha = 1.083$ compares well with our recently published separation selectivity of aspartate and isoaspartate containing peptides of $\alpha = 1.067$ (SD = 0.018).[20] A clear separation of the citrullinated peptide was obtained due to the conversion of a positively charged arginine residue into the uncharged amino acid citrulline.

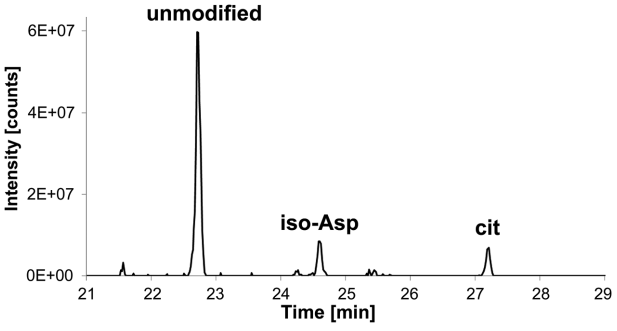

Figure 1. Extracted ion electropherogram of MBP peptide 13–24 from adult mouse. The peptide (13LATASTMD*HAR*H24) exhibits isomerized D20 and citrullinated R23. CE–MS conditions: neutrally coated capillary; BGE, 0.1 M acetic acid; MS instrument, Thermo Scientific Q Exactive HF.

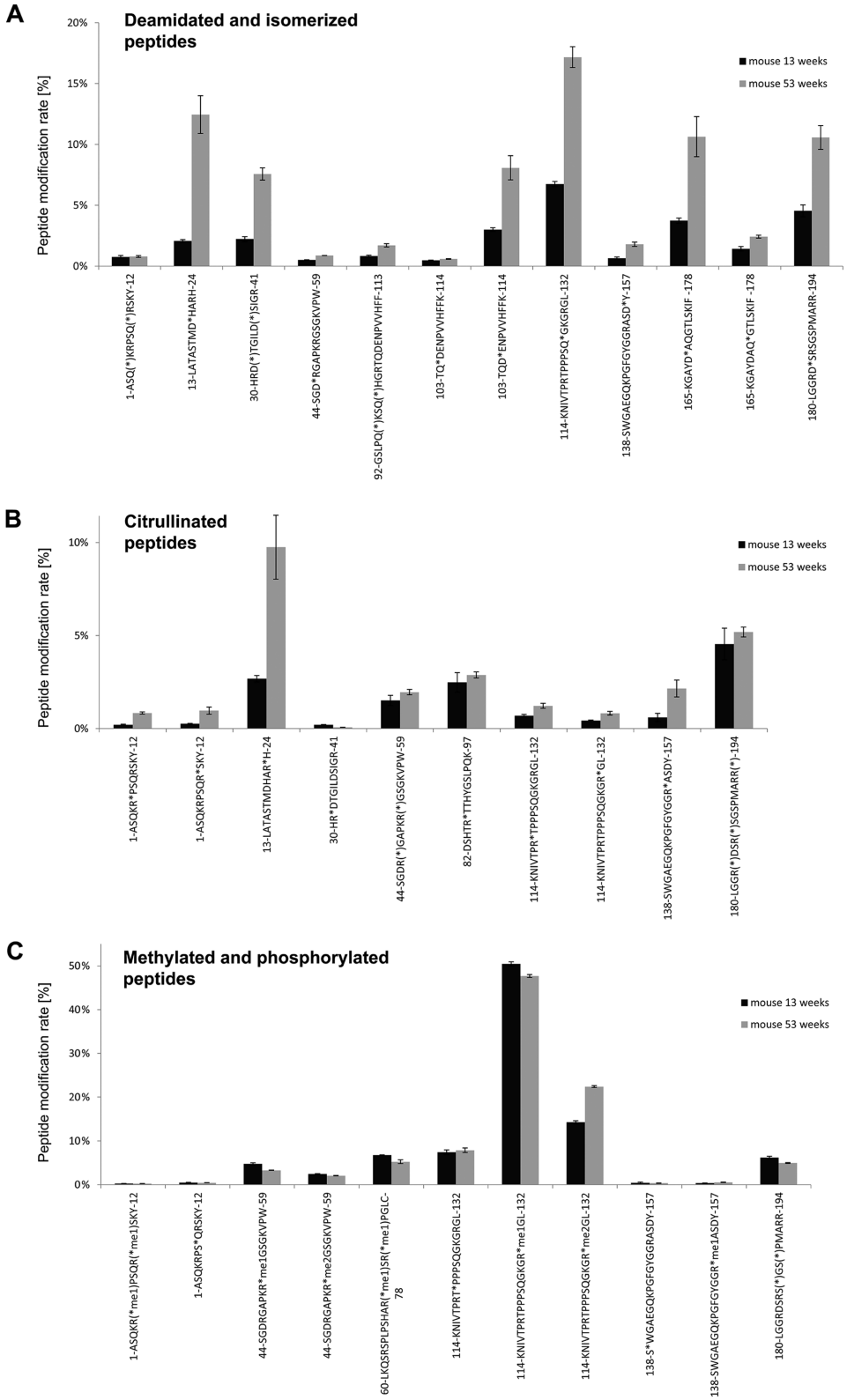

Figure 2. Histogram of the percentage modification at particular sites for mice aged 13 (black) and 53 weeks (gray). The asterisk indicates the site of modification; parentheses indicate undistinguishable modification sites; me1 = monomethylation, me2 = dimethylation. A) deamidated and isomerized peptides; B) citrullinated peptides; C) methylated and phosphorylated peptides. For each enzymatic digest triplicate CE–MS analysis were performed.

A quantification of these modifications in young and adult mice showed a clear age-dependent increase for both modifications (histogram of the percentage modification at particular sites for mice aged 13 and 53 weeks; **Figure 2**A and B).

Many amino acid residues in MBP were found to be modified, and in some cases more than one modification was detected in a single chymotryptic peptide. This is illustrated for MBP (114KNIVTPR**T***PPPS**Q***GKG**R***GL132), where, along with deamidation of Q126 and phosphorylation of T121, R130 was found to be mono- and dimethylated (**Figure 3**). The major peak at 16 min was the unmodified peptide (dark blue), followed by the highly monomethylated (red) and, to a minor degree, dimethylated (dark green) form. Only a minor degree of deamidation of these forms (Q → E + iso-E) was detected. Moreover, strong signals for the phosphorylated nonmethylated (light blue) and phosphorylated monomethylated (yellow) peptides were obtained, with the phosphorylated dimethylated form present to a minor degree (light green). The citrullinated forms have the same migration time as the phosphorylated ones, but due to their different molecular masses they can be clearly differentiated by MS.

The quantification of these modifications revealed that citrullination and phosphorylation were slightly increased in adult mice (Figure 2B and C). The amount of the unmodified and monomethylated peptide in adult mice was similarly decreased while a simultaneous increase of the dimethylated form (Figure 2C) was noted. A strong increase was found in deamidation of Q126 (Figure 2A). A separation selectivity $\alpha = 1.070$ was calculated for the isomerized forms. The ratio between isoglutamate/glutamate is roughly 3.9:1 in young mice and 4.8:1 in adult individuals. Another example for a differently modified peptide is MBP (138 **S***WGAEGQKPGFGYGG**R***AS**D***Y157), which was found to be phosphorylated on S138, isomerized on D156, and either monomethylated or citrullinated on R153. While isomerization and citrullination increase with age, phosphorylation and methylation are unaffected (Figure 2A–C). A similar age-dependent increase in isomerization of D46 and citrullination of R47 was found for MBP (44SG**D***R***R***GAPK**R***GSGKVPW59). The peptide was also found to be mono- and dimethylated on R52, which is a novel MBP modification site. Both mono- and dimethylation slightly decrease in adult brain (Figure 2C).

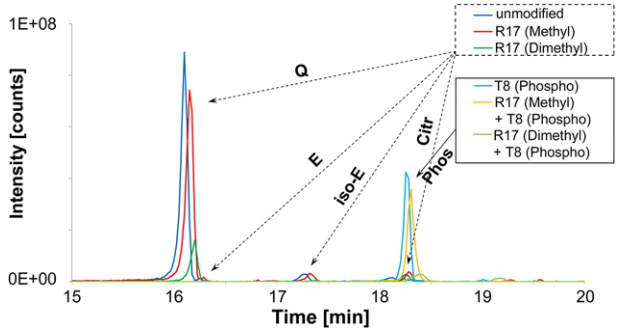

Figure 3. Extracted ion electropherogram of MBP peptide 114–132 from adult mouse. The peptide (114KNIVTPRT*PPPSQ*GKGR*GL132) exhibits phosphorylated T121, deamidated Q126, and mono- and dimethylated R130. Unmodified peptide (dark blue), monomethylated (red), dimethylated (dark green), phosphorylated nonmethylated (light blue), phosphorylated monomethylated (yellow), and phosphorylated dimethylated (light green). CE–MS conditions as described in Figure 1.

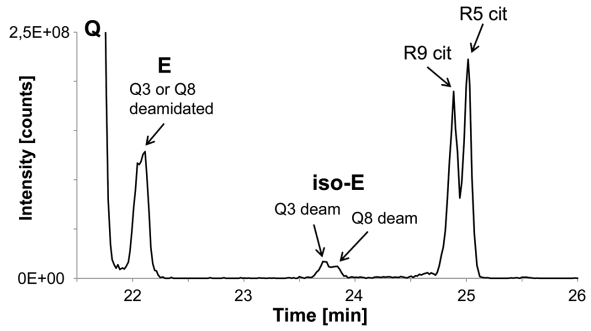

Figure 4. Extracted ion electropherogram of N-terminal MBP peptide 1-12 from adult mouse. The peptide (1ASQ*KR*PSQ*R*SKY12) exhibits deamidated Q3 and Q8 and citrullinated R5 and R9. CE–MS conditions as described in Figure 1.

3.3. Identification of Positional Isomers of Chymotryptic Peptide 1-12

The N-terminal region of MBP was found to be multiply modified, including Nα-terminal acetylation, phosphorylation, deamidation, monomethylation, and citrullination (Supporting Information Table 2A). The extracted ion electropherograms of the deamidated and citrullinated forms revealed that the method enables even a partial separation of different positional isomers. The N-terminal MBP peptide (1**A***S**Q***K**R***P**S***Q***R***SKY12), for example, was found to be deamidated either on Q3 or Q8 and citrullinated on R5 or R9 (**Figure 4**). Based on the ability of CE to separate the isobaric forms, an unambiguous identification of the corresponding modification sites was achieved. Both glutamines were deamidated to a similar extent, however, exact quantification of each site was not possible because of the partial separation. A separation selectivity $\alpha = 1.075$ was calculated for the isomerized forms. The ratio between isoglutamate/glutamate is roughly 0.3:1 in mice aged 53 weeks. Each modification at the N-terminal peptide 1-12 was present in very low amount, for example, in adult mice only 0.8% of the peptide is deamidated, roughly 1% citrullinated, and only 0.3% methylated (Figure 2A–C). The detected methylation site is new, but could not be distinguished between two possible amino acids, R5 and R9. An age-dependent alteration could only be detected for the citrullinated forms (Figure 2B).

3.4. CE–MS Analysis of Lys-C and Trypsin Digested MBP

We performed endoproteinase Lys-C and trypsin digests of the myelin fraction to search for further modifications in MBP. Although overall sequence coverage of MBP and number of modifications identified was less than using chymotrypsin, additional modification sites could be identified (Supporting Information Table 5 and 6). For example, using Lys-C the detection of C-terminal MBP modification was improved. In addition to oxidized M191 and phosphorylated S189 identified

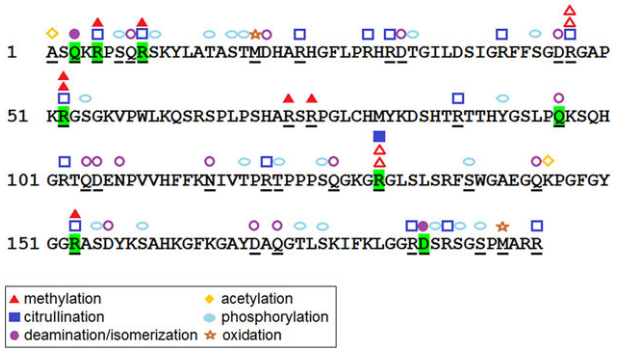

Figure 5. Summary of significant sites of modification of myelin basic protein. The data have been adopted from Harauz and Boggs[3] with addition of more recent data.[26] The figure shows a synopsis of results from different species of different age and of healthy and diseased states (by similarity). Amino acid sequence corresponds to full-length 21.5-kDa classic MBP isoform 4 (UniProt accession number P04370-4).
Novel sites: filled icons + amino acid marked green, detected in this study: amino acids underlined.

by chymotryptic digestion, MBP (180LGG**R***D*SRSG-**S***P**M***AR**R***194) was found to be citrullinated on R183 and R194. Moreover, isomerization of D184 was detected. Both citrullination and isomerization were clearly increased in adult individuals **(**Figure 2A and B**)**. Furthermore, in MBP (83DSHT**R***TTHYGSLPQK97) citrullinated R87 was identified and showed an age-related increase as well **(**Figure 2B**)**. Treatment with trypsin enabled the identification of four further modifications on four different sites, including citrullinated R31, deamidated Q104, and isomerized D32 or D37 and D105. MBP (30H**R***D*TGIL**D***SIGR41) was citrullinated on R31, albeit to a very low amount, both in young and adult mouse. Isomerization was also found on D32 or D37 and showed a clear age-related increase (Figure 2A and B). A clear assignment to one of the two possible sites was not achieved. A further isomerization site on D105 was identified unambiguously in MBP (103TQ*D*ENPVVHFFK114), which was also clearly increased in older mice **(**Figure 2A and B).

A summary of significant modification sites of myelin basic protein is shown in **Figure 5**. The data has been compiled from Harauz and Boggs[3] with addition of more recent data from Friedrich et al.[27] The figure illustrates results from different species of different age and of healthy and diseased states comprising 53 modifications at 50 sites. By combining the CE–MS results we have obtained from chymotrypsin, trypsin, and Lys-C digestion, we were able to add seven new modifications to the known modifications of MBP. The novel sites comprise methylation on R5/R9, R52, R72/74, and R153, deamidation on Q3, isomerization of D184, and citrullination on R130. In total, in healthy mice of different age we identified 40 modifications at 33 different sites.

4. Discussion

In this study we applied CE–MS using a neutrally coated capillary for in-depth analysis of enzymatically digested MBP purified from mouse brain. A large number of modification sites known from human MBP were experimentally proved on mouse MBP for the first time.[27,28]

Of particular interest is the identification of four new methylation sites on MBP. Three of them (monomethylated R5/R9, mono- and dimethylated R52, and monomethylated R153) were found on arginines that can also be citrullinated. It is suggested from previous reports that arginine methylation prevents citrullination at the same residue.[29,30] We were also able to identify a new citrullination site on R130, a site that can be heavily mono- and dimethylated as well. MBP methylation is a process associated with myelination and maintenance of myelin integrity, while citrullination has been implicated in myelin instability and demyelination in multiple sclerosis. Understanding the interplay of multiple arginine modifications and studying the enzymes responsible for these modifications is an emerging area of interest in medical science.[31]

Quantification of methylation sites revealed minor age-dependent alterations with one exception, R130 monomethylation decreased in adult mice with a simultaneous increase of the dimethylated form. This modification site has been found in many species such as human, bovine, rabbit, and chicken. In human MBP, this site was more highly methylated in multiple sclerosis patients than in healthy individuals and has been suggested to play an important role in the pathogenesis of the disease.[28]

Several glutamine deamidations and aspartate isomerizations could be identified and quantified in mouse brain with levels significantly higher in mice aged 53 weeks than in mice aged 13 weeks. These non-enzymatic modifications have been termed degenerative protein modifications,[32] in contrast to enzymatically induced PTMs. Recently, MBP and other proteins in myelin were shown to be long lived.[33] Their gradual degradation over time is associated with the age-related decline of organs and tissues, and contributes to age-dependent defects and many neurological diseases.[34,35] Recently, Friedrich et al. investigated the isoaspartate content of controls and multiple sclerosis patients of different age [27]. Although they analyzed human MBP, some results obtained from their control samples are very similar to our mouse results, e.g. high levels of isomerization on D20 (human D22), on D32 (human D34), and D169 (human D145), respectively.

The isomerized forms could be assigned because of the shift in migration time from aspartate to isoaspartate and glutamate to isoglutamate, respectively. The calculated separation selectivity compared well with our recently published data of aspartate and isoaspartate containing peptides of $\alpha = 1.067$ (SD = 0.018).[20] Glutamate and isoglutamate showed the same migration behavior as aspartate and isoaspartate. Most of the deamidated glutamine sites identified were modified to a very low extent. From two peptides, peptide 1–12 and 114–132, we were able to calculate the isoglutamate/glutamate ratio. It was found to be roughly 3:1 for the N-terminal peptide 1-12, which is in agreement with data recently published by Serra et al. using long-length electrostatic repulsion-hydrophilic interaction chromatography–MS/MS.[36] They suggest that the low amount of isoglutamate is due to transamidation caused by transglutaminases. For most glutamine deamidation products, they obtained an isoglutamate/glutamate ratio of 1:1.7 ± 0.1. Our quantification of MBP 114-132 revealed an isoglutamate/glutamate ratio of 3.9:1 in young and 4.8:1 in adult mouse brain. This result is in

stark contrast to the results of Serra et al., and further investigations will be necessary to elucidate the biological relevance of these results; as to our knowledge, no other studies are available for comparison.

Citrullination was detected at 11 sites and levels were found to be increased significantly in an age-dependent manner. Citrullination of MBP reduces the interaction with the negatively charged phosphatidyl-serines of the myelin sheath due to the loss of basic residues. The ratio of citrullinated MBP/total MBP is crucial in the physiological function of the CNS.[37] Increasing citrullination can cause disruption of the myelin sheath and therefore may contribute to cognitive impairment, such as that observed during the normal aging process.[38] Citrullination adds 0.984 Da to the mass of the intact molecule, which is the same mass increase as observed during deamidation. CE–MS enables the separation of citrullinated and deamidated forms from each other and from the corresponding nonmodified peptides, which is crucial for unambiguous distinction by MS.

Our group recently showed that CE–MS also allows confident assignment between positional isomers, still a very challenging task in the proteomics field.[20] In MBP the N-terminal peptide 1-12 was found to be deamidated on Q3 or Q8 and citrullinated on R5 or R8. The modified forms can be confidently distinguished because of their different migration time. If such positional isomers cannot be separated, an unambiguous assignment of the modification site is problematic, and quantification often impossible. The separation of the positional isomers obtained by CE is most likely the reason why we were able to identify the deamidated Q3 as a new modification site in MBP. In addition, it is challenging to characterize modifications located in close vicinity to each other. There are several regions of the MBP sequence that can be multiply modified, e.g. the N-terminal and C-terminal MBP domains. This resembles the complicated, combinatorial, and dynamic 'histone code', known to be directly linked to the compaction of eukaryotic chromatin, which determines its accessibility to transcription and replication factors.[39] The formation and compaction of the myelin sheath could also be affected by dynamic modifications of MBP.[40] Understanding the interplay of multiple modifications is an emerging area of importance in health and disease and can be best achieved through the development and application of novel tools, with CE–MS very likely among of them.

Abbreviations

BGE, background electrolyte; CESI, integration of capillary electrophoresis and electrospray ionization into a single device; MBP, myelin basic protein; PTM, posttranslational modification

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors have declared no conflict of interest.

Keywords

capillary electrophoresis–mass spectrometry, citrullination, deamidation, isomerization, myelin basic protein

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