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Myelin-derived and putative molecular mimic peptides share structural properties in aqueous and membrane-like environments

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Abstract

Background: Despite intense research, the causes of various neurological diseases remain enigmatic to date. A role for viral or bacterial infection and associated molecular mimicry has frequently been suggested in the etiology of neurological diseases, including demyelinating autoimmune disorders, such as multiple sclerosis. Pathogen mimics of myelin-derived autoimmune peptides have been described in the literature and shown to induce myelin autoimmune responses in animal models.

Methods: We carried out a structural study on myelin-derived peptides, and mimics thereof from various pathogens, in aqueous and membrane-like environments, using conventional and synchrotron radiation circular dichroism spectroscopy. A total of 13 peptides from the literature were studied, and 290 circular dichroism spectra were analysed. In addition, peptide structure predictions and vesicle aggregation assays were performed.

Results: The results indicate a high level of similarity in the biophysical and folding properties of the peptides from either myelin proteins or proteins from pathogenic viruses or bacteria; essentially all of the studied peptides folded in the presence of lipid vesicles or under other membrane-mimicking conditions, which is a sign of membrane interaction. Many of the peptides presented remarkable similarities in their conformation in different environments.

Conclusions: As most of the studied epitope segments in myelin proteins are associated with membrane-binding sites, our results support a view of molecular mimicry, involving lipid membrane interaction propensity and similar conformational properties, possibly playing a role in demyelinating disease. The results suggest mechanisms related to protein amphiphilicity and order-disorder transitions in the recognition of peptide epitopes in autoimmune demyelination.

Keywords: Myelin, Peptide, Folding, Lipid membrane, CD spectroscopy, Synchrotron, Molecular mimicry, Structure

Background

Myelin is a multilayered, tightly packed proteolipid membrane structure crucial for the rapid saltatory conduction of nerve impulses in vertebrates. It is formed when the plasma membrane of a myelinating glial cell wraps around an axon, with eventual compaction driving out excess cytoplasm and extracellular fluid. Demyelinating diseases are neurological conditions related to the

loss of the myelin sheath around axons. Such diseases can be caused by inherited mutations, often concerning myelin-specific proteins, or autoimmune reactions against myelin components. For most demyelinating diseases, the corresponding molecular mechanisms remain unknown, directly impairing our understanding of these disorders and their potential treatment.

Multiple sclerosis (MS) is a central nervous system (CNS) demyelinating autoimmune disease with a complex, incompletely known etiology. The current understanding of the pathogenesis and immunological models of MS has been reviewed [1, 2]. MS is an autoimmune

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disease with an initial autoreactive, T cell-mediated mechanism, while B cell-expressed autoantibodies likely play a role in disease progression. Several peptide epitopes from myelin proteins, including myelin basic protein (MBP) [3], proteolipid protein (PLP) [4, 5], myelin-oligodendrocyte glycoprotein (MOG) [6], and myelin-associated oligodendrocytic basic protein (MOBP) [7, 8] have been implicated as T cell epitopes in MS, and many such peptides have been used to induce experimental autoimmune encephalomyelitis (EAE), the most widely used animal model of MS [9]. In addition, the myelin proteins P0, P2, and PMP22 have similarly been implicated in the peripheral nervous system (PNS) autoimmune disease Guillain-Barré syndrome (GBS) and its corresponding animal model, experimental autoimmune neuritis (EAN) [10–12]. In GBS, a common disease-preceding infection is by *Campylobacter jejuni* [13, 14].

It is unclear, how autoreactive T cells are triggered, but environmental factors play a significant role in MS. Especially inflammations caused by viral or microbial infections have been implicated. For example, Epstein-Barr virus infection seems to correlate with MS morbidity [2]. A number of other viral pathogens have also been implicated in MS etiology [15–18]. The proposed immunological mechanisms behind infection-induced autoimmunity include molecular mimicry, bystander activation, and dual-specificity T cell receptor (TCR) expression. While in the latter two mechanisms, the response towards the infective agent either indirectly or co-operatively induces autoreactive T cell function, molecular mimicry predicts immunological identity or close similarity between the sequences and/or structures of self-epitopes and viral/bacterial proteins at the molecular level.

Several viral and bacterial peptides harbour structural epitopes that mimic the well-characterized immunodominant MBP85-99 peptide, albeit they share very limited sequence similarity, with only a few conserved amino acids required for major histocompatibility complex (MHC) or TCR binding [19]. However, it is not known whether the self-epitopes are present in the native physiological state, or whether they form after conformational changes or denaturation caused by insults against myelinating glial cells and subsequent antigen presentation. MBP isolated from nerve tissue has been for long known for its intrinsically disordered structure and conformational changes induced by lipids [20–27]; much of this work stems from isolation of native protein from tissue, which will inevitably produce heterogeneous samples. Full-length 18.5-kDa murine MBP and the MBP72-107 peptide underwent a disordered-to-ordered transition, when transferred from an aqueous to a membrane-like environment in a trifluoroethanol (TFE) titration experiment [28, 29]. We observed folding of recombinant

18.5-kDa MBP in various membrane-mimetic conditions, as well as in the presence of lipid vesicles [30]. Similarly, the MBP85-99 peptide acquired helical conformation in the presence of detergent micelles and lipid vesicles [31]. The main immunogenic epitope of MBP, represented by the MBP85-99 peptide, corresponds to the central membrane attachment site, and correlations between the strength of the membrane interaction and MS etiology have been suggested [32]. Taken into account recent theories on the formation of a protein phase consisting of MBP upon myelin membrane maturation [33], it is clear that membrane binding and related conformational changes in MBP are crucial for normal myelin formation and maintenance.

The above findings raise questions on whether molecular mimicry in the context of MS may focus on structural epitopes, which change during water-lipid environmental transitions. Importantly, conformation-specific anti-MOG antibodies are required for MS-type pathological changes in EAE [34]. Therefore, there exists significant interest to find out, whether the autoimmunogenic epitopes in myelin proteins undergo conformational changes, when shifted between aqueous and hydrophobic, membrane-like environments, and whether the pathogen epitopes, which share similar T cell recognition, behave similarly. Could similar behaviour of peptide epitopes from myelin proteins and putative pathogenic mimics in membrane-mimicking conditions be correlated to autoimmunogenic properties in the specific case of demyelinating autoimmune diseases?

Here, circular dichroism (CD) spectroscopy was used to follow conformational changes in several myelin self-epitope peptides from PLP, MOBP, MOG, and P2 and in four MBP85-99-mimicking viral/bacterial peptides in aqueous and membrane-like environments. All peptides were picked from relevant literature. We observed that the autoimmunogenic candidate peptides underwent folding, when shifted from an aqueous to a hydrophobic environment. The same change was evident for the MBP-mimicking peptides. All the results point towards shared biophysical and structural properties in the different peptide epitopes.

Methods

Peptides

Synthetic peptides were from GenScript, Piscataway, NJ, USA. Details of the peptides are given in Table 1. Peptides were dissolved in sterile water to get 10 mg/ml stock solutions. All peptides were acetylated at the N terminus and amidated at the C terminus.

Detergents and lipids

n-Dodecyl phosphocholine (DPC) was from Anatrace and sodium dodecyl sulfate (SDS) from Sigma. Dimyristoyl

Table 1 The peptides selected for the current study

Peptide	Sequence	Predicted structure	References
MOBP15-36	QKFSEHFSIHCCPPFTFLNSKR	beta/disordered	[7]
MOBP37-60	EIVDRKYSICKSGCFYQKKEEDWI	alpha/disordered	[8]
hMOG35-55	MEVGWYRPPFSRVVHLYRNGK	beta/disordered	[6]
mMOG35-55	MEVGWYRSPFSRVVHLYRNGK	beta/disordered	[6]
HSV-mimic	FRQLVHFVRDFAQLL	alpha	[19]
EB-mimic	TGGVYHFVKKHVHES	alpha	[19]
IA-mimic	YRNLWFIKKNTRYIP	beta/disordered	[19]
PA-mimic	DRLLMLFAKDVS RN	alpha	[19]
P2gbs	ENFDDYMKALGV	alpha/disordered	[78]
PLP139-151	VSLGKWLGHDPKF	alpha/disordered	[4]
PLPextra2	FNTWTCQSIAFPSKTSASIGSLCADARMYG	alpha/disordered	[5, 60]
PLPintra1	AEGFYTTGAVEQIFGDYKTTICGKLSATVTGGQKGRG	mixed	[60, 100]
PLPintra2	SRGQHQAHS LERVCHCLGKWLGHDPK FVGI	alpha/disordered	[4, 60]

The predicted structure corresponds to that obtained from PEP-FOLD. References for the possible disease linkage of each peptide are also given

phosphatidylcholine (DMPC) and dimyristoyl phosphatidylglycerol (DMPG) were from Affymetrix. To prepare lipid vesicles, the lipids were dissolved in 1:1 methanol:chloroform. The lipid solutions were then mixed in a 1:1 molar ratio, and solvent was evaporated under a gentle air stream. The dried lipid mixtures were suspended to 10 or 20 mg/ml in water or 10 mM HEPES (pH 7.5). Hydration was completed and unilamellar vesicles prepared by sequential sonication (30 min Branson 3510 bath sonicator, followed by 60 s Branson 450 ultrasonifier with 10% power and a 1 s pulse–1 s chase protocol).

Circular dichroism spectroscopy

Environment-induced folding of peptides was analyzed by CD spectroscopy, using a Chirascan Plus spectropolarimeter (Applied Photophysics). Quartz cuvettes with a 1-mm light path were used to measure CD spectra from 260 to 190 nm. The samples were water-dissolved peptide samples of nominal concentrations of 50 to 100 µg/ml, with or without TFE, detergents, or lipids (added immediately before the measurement). A peptide from the *Plasmodium falciparum* formin was used as a negative control. Synchrotron radiation CD (SRCD) spectra were recorded at +30 °C on the DISCO beamline at the SOLEIL synchrotron (Paris, France) and on the AU-CD beamline at the ASTRID2 storage ring (ISA, Aarhus, Denmark), using 0.2- and 0.5-mm quartz cuvettes and 0.25 mg/ml peptide.

In the oriented CD (OCD) experiment, 200 µl of a peptide-lipid (1:30 molar ratio) mixture, containing 10 µg of peptide, or 200 µl of a DMPC:DMPG (1:1) lipid blank alone, were pipetted onto a quartz disk. The generation of multilamellar planar lipid bilayers was conducted by air-drying, followed by 10 h of re-hydration in a saturated K₂SO₄ atmosphere at +30 °C (relative

humidity 97%) [31]. OCD spectra were recorded with 45° rotation steps on the DISCO beamline at the SOLEIL synchrotron (Paris, France), and the resulting eight spectra, covering 360° of total rotation, were averaged. Due to the rather harsh sample preparation procedure and the tendency of the peptides to aggregate membranes, only the EB-mimic peptide gave a high enough signal for further analysis.

SRCD and OCD spectra were averaged and backgrounds subtracted with CDtool [35]. A total of 290 CD spectra were measured and analysed.

Structure prediction and CD deconvolution

De novo structure prediction of the peptides was done *in silico* using the PEP-FOLD server [36, 37]. Membrane association modes of protein and peptide models were predicted using PPM [38]. Secondary structure contents were inferred by deconvoluting the CD spectra with the DICHROWEB server using the CONTINLL algorithm with DICHROWEB reference databases 6 and 7 for SRCD and regular CD, respectively [39]. The Bestsel server [40] was also used. It should be noted that in the case of short peptides, deconvolution algorithms optimized for full proteins give semi-quantitative or qualitative results, and we think more information is available by simply observing and comparing the full spectra, instead of relying on numbers from deconvolution. Spectra of short peptides are strongly affected by amino acid composition, for example.

Turbidimetry

Peptide-induced vesicle agglutination was studied in 0.5 mM DMPC:DMPG lipid in water. Peptides or proteins were added at the start of the experiment, and the

experiment was carried out essentially as described before [41]. The full-length human P2 protein was used as a positive control [41]. Another possible positive control could have been full-length MBP [42].

Turbidity changes as a function of peptide concentration were followed by absorbance measurement at 450 nm in 96-well format using a Tecan Infinite M1000 Pro plate reader (Tecan, Salzburg, Austria). The total volume was 150 μ l, and 2 s orbital shaking with 1 mm amplitude and 150 ms settling time were used.

Results

Demyelinating autoimmune diseases, such as MS or GBS, are thought to present a molecular mimicry component related to relatively common viral or bacterial pathogens, many of which involve the nervous system in their infection. This research was sparked by our interest in studying putative common biophysical properties between myelin-derived autoimmune peptides and peptides from pathogens described in the literature and proposed to cause molecular mimicry in e.g. multiple sclerosis. Our hypothesis was that such peptides might share similar properties with respect to structure, conformational changes, and lipid membrane binding, and furthermore, that peptides affecting the CNS and PNS might have similar properties.

Myelin-derived or myelin-mimicking peptides of potential relevance to demyelinating disease

Nine peptides from four human or mouse compact myelin proteins with a putative autoimmunogenic character and four viral/bacterial peptides suggested [19] to mimic the well-known MS-associated MBP sequence (amino acids 85–99) were chosen for structural analysis; all peptides have been described in the literature earlier (see Table 1 and references therein). The corresponding MBP85–99 peptide was a subject of our earlier study [31]. An additional common denominator of the myelin protein peptides was known or suggested membrane contact. The N terminus of MOBP is predicted to have a membrane-associated FYVE domain [43]. The peripheral myelin protein P2 acts in the formation of myelin membrane stacks, with the two α helices involved in membrane contacts [41, 44]. MOG and PLP are transmembrane proteins, in which both the extracellular and cytoplasmic domains may interact with the next opposing lipid bilayer. In PLP, the autoimmunogenic peptides are in the close vicinity of the transmembrane domains, forming loops between them. Finally, pathogen-derived peptides were predicted to mimic the autoimmunogenic central membrane-anchoring segment of MBP, which has been shown to become embedded into the lipid bilayer [19, 31, 45–49].

Based on amino acid sequences, the peptides do not exclusively share obvious biophysical characteristics. However, most of the peptides are positively charged at neutral pH and contain hydrophobic amino acids, which may be important for membrane attachment (Additional file 1: Table S1). The charge distributions are evenly scattered along the sequence. However, most peptides showed amphipathic distribution of positively charged and hydrophobic residues on helical wheel models (Fig. 6b, Additional file 2: Figure S1), possibly reflecting propensity for interactions with phospholipid membrane surfaces. A predicted property for most, but not all, of the studied peptides is the presence of a membrane-induced amphipathic helix.

The peptide *de novo* structure was predicted *in silico* using the PEP-FOLD algorithm [36] (Additional file 3: Figure S2). The MOG-derived peptides, as well as the Influenza hemagglutinin peptide (IA mimic), were predicted to harbor β strands, whereas other MBP-mimicking peptides (HSV mimic, EB mimic, and PA mimic) as well as the P2-derived peptide P2gbs, contained an α helix as the dominant predicted secondary structure. The latter corresponds to α helix 1 in the crystal structure of human P2 [41, 44]. Peptides from PLP were predicted to consist of α -helical parts with a β strand (PLPintra1) or disordered sequences (Table 1), while MOBP15–36 and MOBP37–60 were predicted to have β strand and α helix, respectively, in addition to disordered regions (Table 1). While all peptides were experimentally observed to maintain an unfolded structure in aqueous solution, these predicted propensities of folding were, in fact, rather consistent with CD spectroscopic experiments under membrane-mimicking conditions (see results below).

During the formation of compact myelin, the peripheral membrane proteins of myelin, as well as the extra- and intracellular domains of integral myelin membrane proteins, experience change from an aqueous/single membrane physical environment to a dehydrated membrane multilayer. After mechanical damage, or an inflammation/infection-induced insult to myelin, an opposite environmental change is possible, whereby membrane-embedded epitopes may be exposed to aqueous conditions. CD spectroscopy is a powerful method to probe solvent effects on peptide structure. Previously, it has been shown that many myelin protein-derived peptides, indeed, undergo folding, when shifted from aqueous buffer into a more hydrophobic environment [31, 50]. We monitored the CD spectra of the 13 selected peptides (Table 1) by conventional and SRCD in the presence of different concentrations of TFE, DPC, and SDS, as well as with DMPC:DMPG (1:1) vesicles.

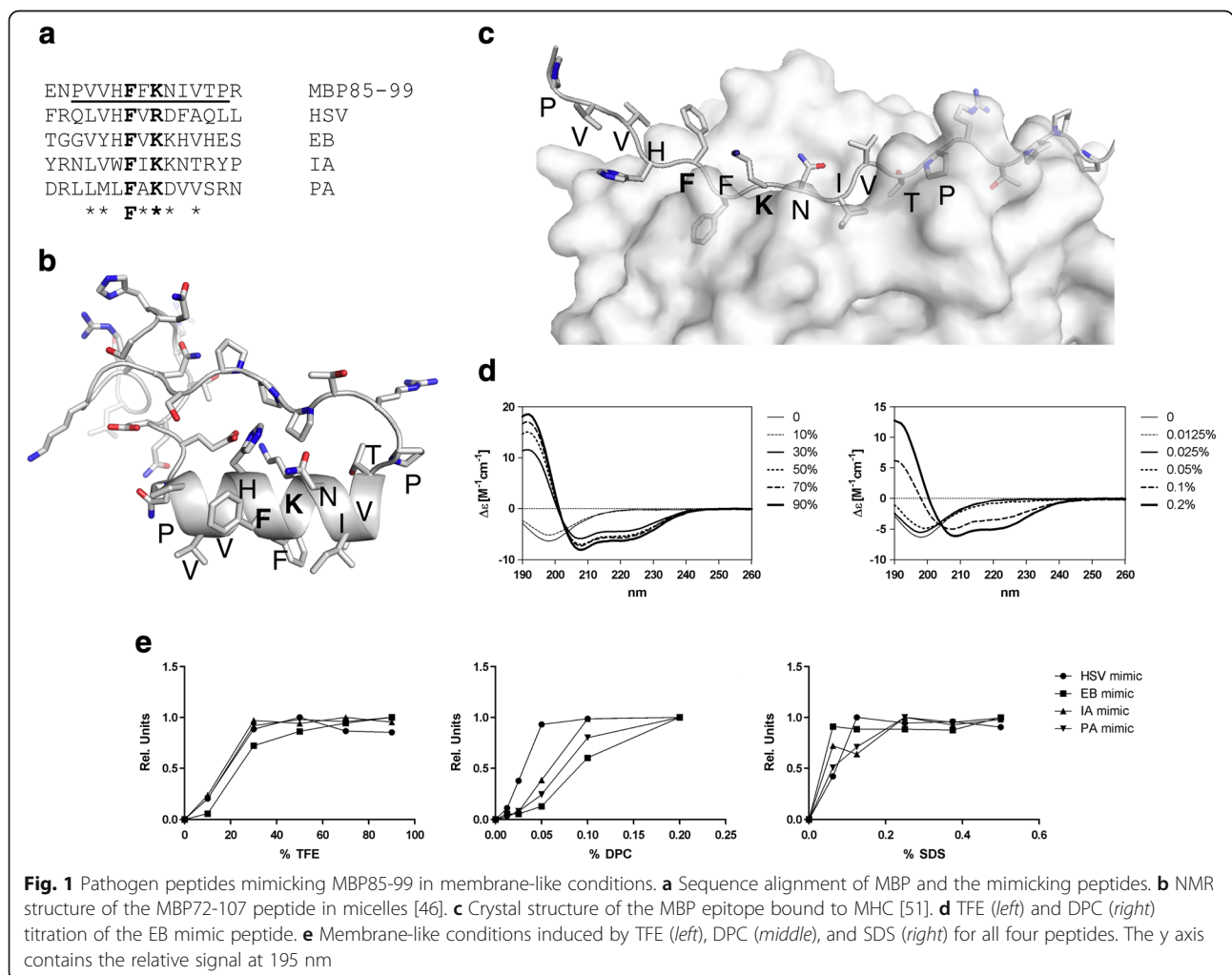
In general, all studied peptides were disordered in water and showed TFE-, SDS- (a negatively charged

detergent, critical micellar concentration CMC = 8.2 mM; ~0.24%), and lipid vesicle-induced folding at variable levels, while DPC (a zwitterionic detergent, CMC = 1.1. mM; ~0.04%) did not induce folding of MOBP peptides (see below for details). Of note, a peptide from the formin of the malaria parasite did not fold upon membrane-mimicking conditions and was considered a negative control (Additional file 4: Figure S3). Consistently, CONTINLL predicted higher degrees of folding and higher α/β ratios than the Bestsel algorithm. However, these differences were minor in terms of quantity. Finally, as expected, the diagnostic value of the wavelength region 180–190 nm, which can be reliably measured only with SRCD, was significant. This could be observed by comparing the secondary structure content deconvolutions between conventional CD (190–260 nm) and SRCD (180–270 nm), as the programs inferred conventional CD spectra in favour of β strand content, which likely arose from random coil structure, as indicated by SRCD data. The results from secondary

structure deconvolution of all 290 CD spectra with both of the algorithms are given in Additional file 5: Table S2.

MBP85-99-mimicking peptides demonstrate a major disorder-to-helix transition induced by TFE, micelles, and lipid vesicles

The four MBP85-99-mimicking peptides (Fig. 1a), ‘HSV-mimic’ from tripartite terminase subunit 3 (UL15) of *Human herpes simplex virus 1*, ‘EB-mimic’ from *Epstein-Barr virus* DNA polymerase catalytic subunit (BALF5), ‘IA-mimic’ from *Influenza A* virus hemagglutinin, and ‘PA-mimic’ from *Pseudomonas aeruginosa* phosphomannomutase (algC), were selected based on earlier literature on their properties related to molecular mimicry in autoimmune response [19]. Structural information for the corresponding segment in MBP is available (Fig. 1b, c), and while it is helical when bound to membranes [31, 47], its conformation is extended when bound to MHC [51]. The residues most conserved between MBP and the mimic peptides lie slightly embedded in the membrane in



the helical conformation, and they point outward from the MHC complex; these are the positions that MHC presents to the T cell. Mutation of these positions in the MBP85-99 epitope lead to altered immunological response [52, 53].

In CD experiments, the mimic peptides were mainly disordered in water and acquired folded structure after moving into membrane-like environments (Fig. 1d, e and Additional file 5: Table S2). This behavior is highly similar to that of the MBP85-99 peptide [31]. From the SRCD spectra of peptide-lipid vesicle mixtures (Fig. 2), CONTINLL and Bestsel, respectively, calculated α -helical contents of 91 and 79% for EB-mimic, 57 and 58% for HSV-mimic, 63 and 62% for IA-mimic, and 43 and 38% for PA-mimic. Interestingly, while the predicted structure *de novo* (Table 1) was helical for EB-mimic, HSV-mimic, and PA-mimic, IA-mimic was predicted to have a β strand, which is also present for this segment in the hemagglutinin protein [54]. Similarly to MOG35-55 (see below), the IA-mimic peptide thus shows, at least as an isolated peptide, that it has a propensity for helical conformation under membrane-like conditions. Such common properties may explain the encephalitogenic properties of many myelin- and pathogen-derived peptides, despite the context of the corresponding sequence in the full protein.

The EB-mimic peptide membrane orientation is similar to MBP85-99

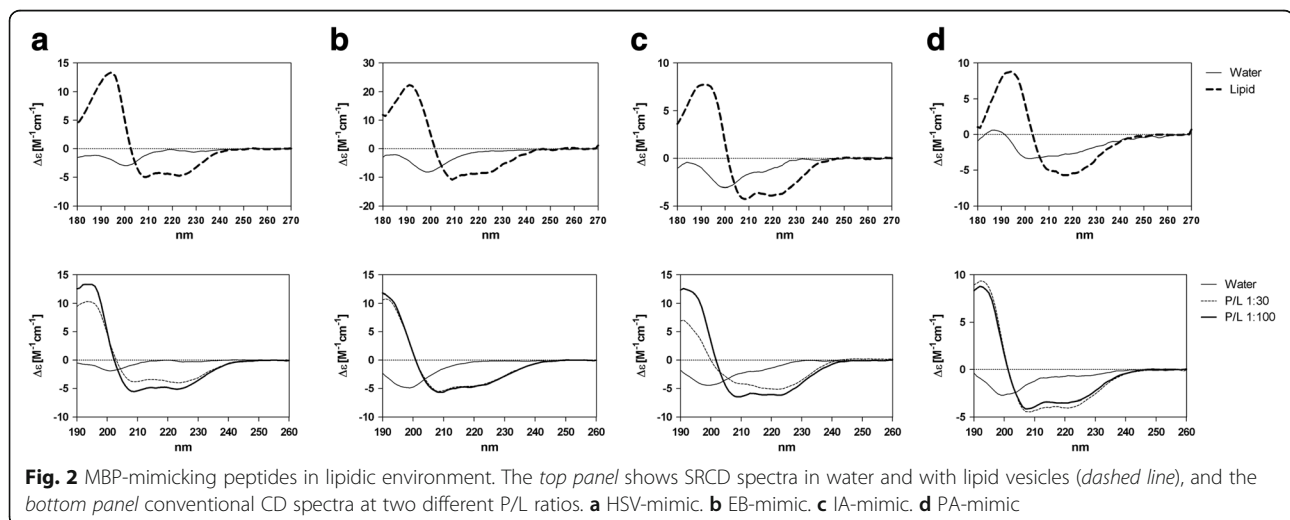
Previously, we [31] have shown by OCD that MBP85-99 is partially embedded into a lipid membrane leaflet in a tilted orientation. We repeated the experiment here for the EB mimic peptide, which had the highest helical content when mixed with vesicles (see above). Briefly, 10 μ g of peptide was mixed with DMPC:DMPG (1:1) vesicles with a 1:30 P/L ratio, and OCD was measured from planar multilayered membranes. The 208-nm

minimum, typical for α -helical conformation, which was observed in isotropic solution CD spectra, was missing in OCD (Fig. 3), indicating either a conformational change or assembly of the peptide in a tilted orientation in the oriented membrane layers. This observation supports the hypothesis that the MBP85-99 self-epitope and the mimic peptides share similar conformational epitopes.

The MOG35-55 epitope has a propensity to adopt helical conformation

The MOG35-55 epitope (Fig. 4a) is a widely recognized inducer of encephalitogenic reactions, and it is routinely used to induce mouse EAE models [55, 56]. The mMOG35-55 epitope is highly immunogenic and induces EAE, while the corresponding peptide from human MOG is less active in causing EAE [57]. This is surprising, since only one residue distinguishes mMOG35-55 and hMOG35-55 from each other.

Both human and mouse MOG35-55 were largely disordered in water and acquired α -helical character in hydrophobic environments (Fig. 4). While these peptides differ only by a single amino acid (Pro/Ser42), the α/β ratio was in general higher for hMOG35-55 in membrane-mimicking conditions (Fig. 4, Additional file 5: Table S2). The SRCD spectra of the two peptides with lipid vesicles also suggest more β structure content for mMOG35-55 (Fig. 4b). In the crystal structure, the peptide sequence covers two β strands and a connecting loop [58] (Fig. 4a), which was also predicted for these peptides *de novo* in aqueous environment (Table 1). However, based on our results, this autoimmunogenic region in MOG has an intrinsic propensity to adopt α -helical conformation in the context of a peptide interacting with membranes. Subtle differences in folding between the mMOG35-55 and hMOG35-55 epitopes may be relevant



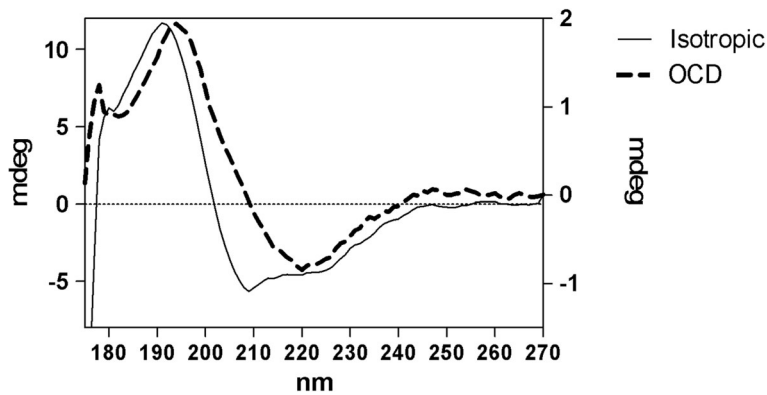


Fig. 3 Orientation of the EB-mimic peptide on a membrane. The figure shows comparison of isotropic and oriented SRCD spectra. The loss of the peak at 208 nm in OCD is a sign of tilting of the peptide with respect to the membrane surface plane

for inducing EAE with MOG peptides. Mutations in the mMOG35-55 peptide have also been linked to altered immune responses in mouse models [59]. Whether the recognition of the Pro/Ser residue itself or the variant-favored conformation determines the strength of autoimmunogenicity remains to be studied.

MOBP peptides fold into different structures in membranes

The N terminus of MOBP, shared by all known MOBP isoforms, is predicted to fold into a FYVE-like membrane interaction domain [43] (Fig. 5a, b). The epitope peptide segments correspond to areas predicted to

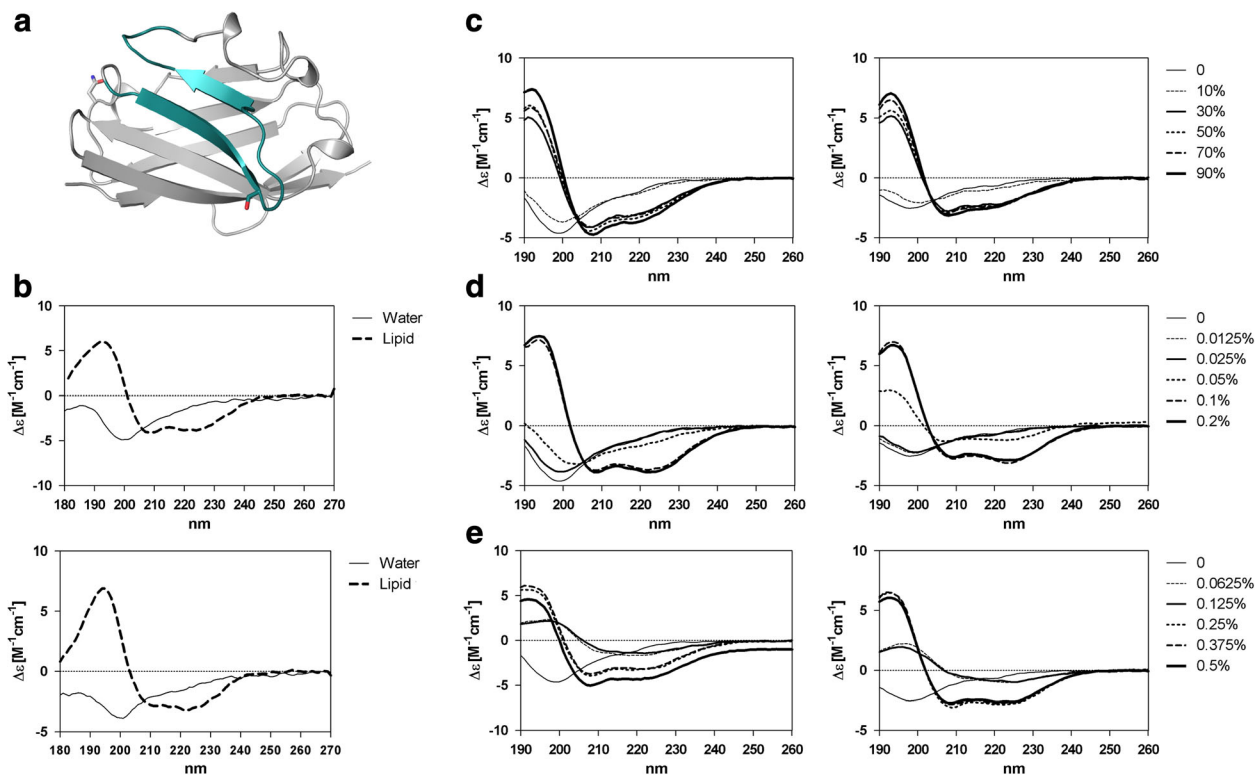
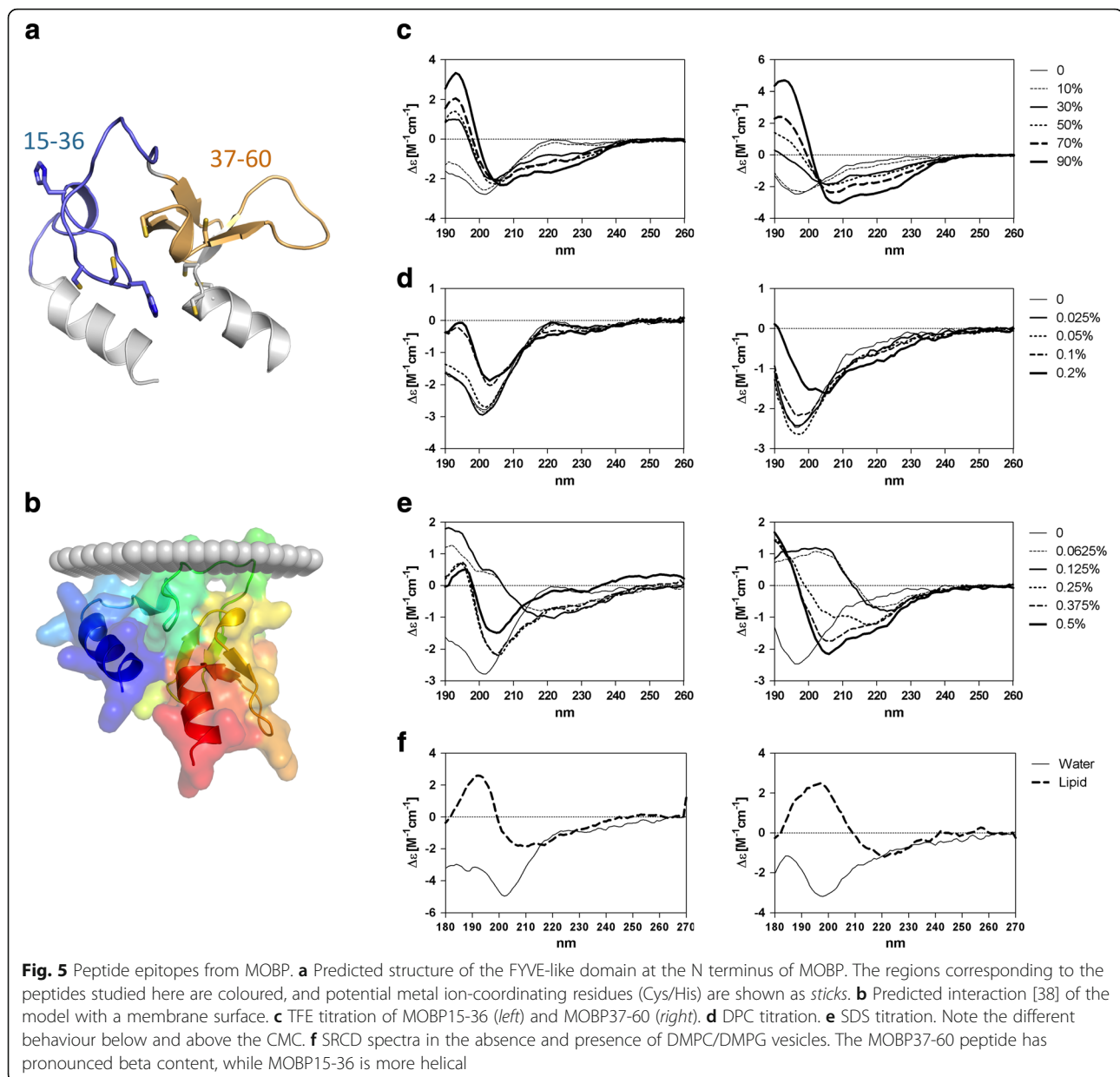


Fig. 4 The MOG35-55 peptides. **a** Location of the segment 35–55 in the MOG Ig domain crystal structure [58]. Shown (as sticks) are also the glycosylation site Asn31 [101] and the Ser42 residue (at the hairpin of the MOG35-55 epitope), which when mutated to Pro, as in human MOG, produces a less encephalitogenic response [57]. **b** SRCD spectra of the human (top) and mouse (bottom) MOG35-55 peptides in the absence and presence of lipid vesicles. Similar results were also obtained in conventional CD at P/L ratios of 1:30 and 1:100. **c** TFE titration of the human (left) and mouse (right) peptides. **d** DPC titration. **e** SDS titration. Note the different behaviour of both peptides below and above the CMC



closely interact with the lipid membrane surface. So far, no high-resolution structural or functional studies with purified MOBP protein have been carried out. The *de novo* prediction suggested some β and α structures for the MOBP15-36 and 37-60 peptides, respectively, with disordered regions (Table 1), but in contrast to the MBP-mimicking and MOG35-55 peptides, models predicted by PEP-FOLD were not always consistent.

Both MOBP peptides (15-36, 37-60) showed hydrophobicity-induced folding (Fig. 5b-f). DPC induced some secondary structure formation at high concentrations (Fig. 5d), while both peptides behaved differently in SDS. Below the CMC of SDS, the CD spectra are

reminiscent of β aggregates, while above the CMC, micelles of SDS induce significant helical components (Fig. 5e). The SRCD spectra of lipid vesicle-induced folding suggested a β sheet structure for MOBP37-60, while MOBP15-36 contains more helical structure (Fig. 5f). While these results differ somewhat from the other peptides in this study, which form α -helical structures under most conditions, they are logical and fit to the predicted FYVE domain structure of MOBP [43].

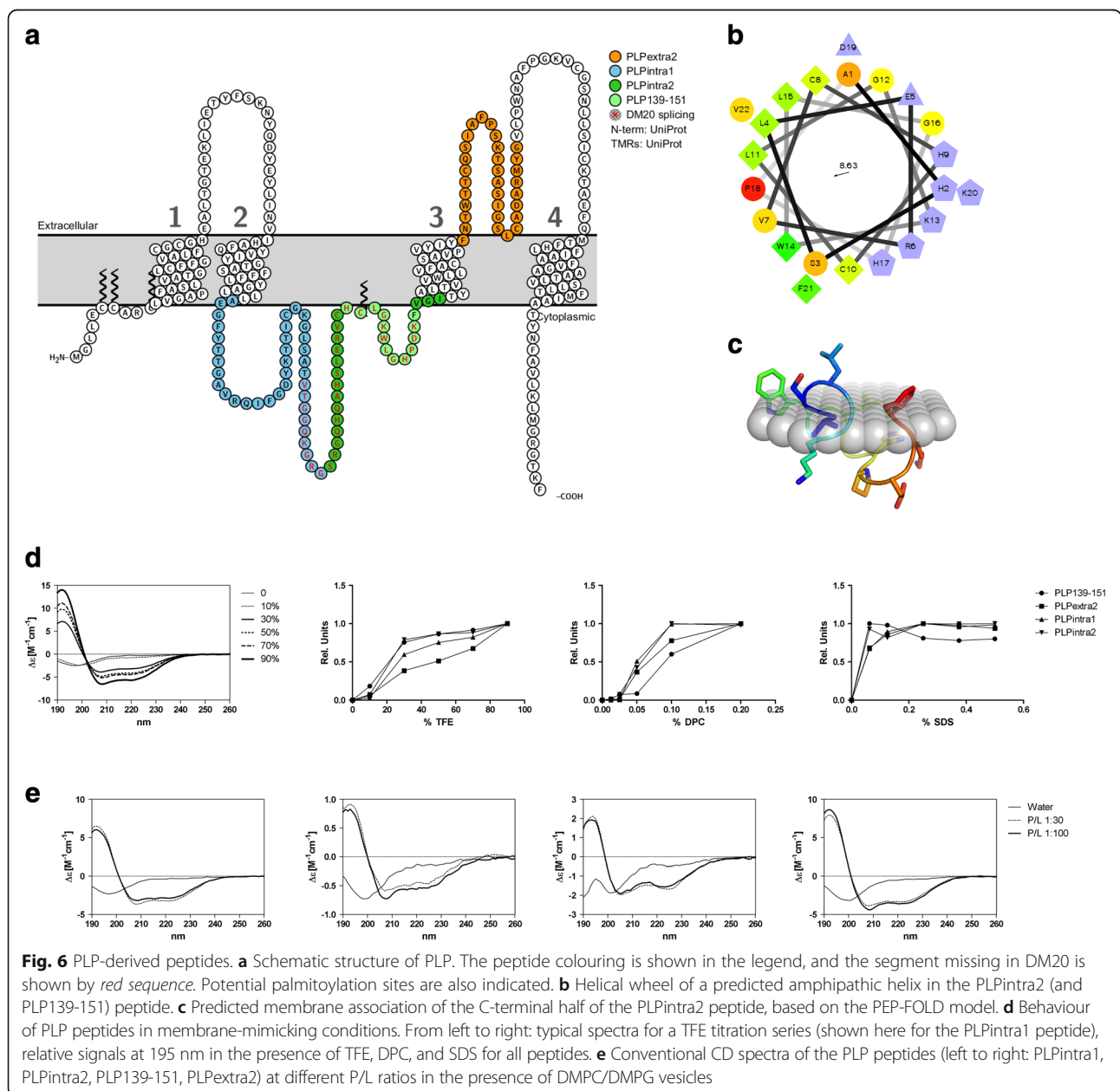
PLP-derived peptides fold in hydrophobic environments

The peptides from PLP were based on earlier studies on EAE models, and included both extra- and intracellular

epitopes (Fig. 6a) shown to induce antibody responses related to demyelination in EAE [60, 61]. In the overlapping peptides PLP139-151 and PLPintra2, a region with strong propensity to fold into an amphipathic helix can be detected (Fig. 6b, c). Remarkably, all the PLP peptides also exhibited folding in membranous environments, although they are expected to represent loops between the transmembrane helices in the PLP protein (Fig. 6d, e). The result strongly suggests that these loops are able to interact directly with lipid membrane surfaces. In the case of compact myelin, this could lead to a single molecule of PLP acting as glue between three successive membrane layers. Interestingly, a C-terminal peptide

from PLP was also recently shown to fold upon membrane interaction, and the folding was affected by peptide concentration, the helices refolding into β sheet structure at high concentration on the lipid membrane – a process, which might play a role in myelin membrane stacking [62].

The peptides PLP139-151 and PLPintra2 overlap, PLPintra2 being an extended version of the widely used encephalitogenic PLP139-151 peptide. Furthermore, the region covered by PLPintra2 corresponds nearly exactly to the segment missing in the alternatively spliced DM20 isoform of PLP. DM20 is believed to be less adhesive in membrane multilayers than PLP [63]. The fact



that peptides corresponding to the region missing in DM20 show clear, strong folding events in membrane-like environments suggests that indeed, a membrane-binding site is removed from DM20 during alternative mRNA splicing. It is likely that this segment folds into an amphipathic helix onto the membrane surface (Fig. 6c); this propensity should be further strengthened by hydrophobic post-translational modifications (palmitoylation) on the two Cys residues, as suggested by a helical wheel representation (Fig. 6b).

The palmitoylation of the Cys residues has been observed to induce greater T cell and antibody responses in both EAE mice and MS patients compared to nonacylated peptides [64, 65]. This further supports the relevance of the hydrophobic surface of an amphipathic helix to the immunogenicity of PLP139-151 and PLPintra2 peptides. We believe that this segment is a strong membrane anchor in PLP. Whether it binds to the same or the apposing membrane surface in myelin, is currently unknown. Both scenarios would explain stronger adhesive properties of PLP in comparison to the DM20 isoform.

During evolution, the 35-residue segment distinguishing PLP from DM20 has been acquired in terrestrial vertebrates [63, 66]. Simultaneously, the expression of myelin protein zero (P0) in the CNS has been lost. This points towards a scenario, where the evolution of terrestrial vertebrates at the myelin molecular level has involved the loss of P0-mediated adhesion (which still is crucial in the PNS) and the acquirement of an additional adhesive segment in the cytoplasmic domain of PLP/DM20. PLP has a strong tendency to form dimers in transfected COS cells, primary oligodendrocytes, and in brain tissue, while DM20 exists mainly as a monomer. Cys108, located in the common part of intracellular loop of PLP/DM20, as well as in the PLPintra1 peptide, is involved in the dimerization of PLP [67].

Both the PLP139-151 and PLPintra2 peptides include a 'stop transfer signal' (residues HPDK¹⁵⁰), which restricts the ability of transmembrane helix 3 to shift in the cases of mutations in the second extracellular loop of PLP. These charged residues are omitted from DM20, enabling the movement of the transmembrane domain inside the membrane [68]. Thus, in some cases, the middle part of the PLPintra1 peptide (residues GLSAT¹¹⁵) forms an extension of transmembrane helix 3 and can be embedded into the membrane. Several PLP139-151 epitope-mimicking peptides, not studied here, are also known e.g. from *Acanthamoeba castellanii* [69] and *Haemophilus influenzae* [70]. Whether they share properties with PLP139-151 remains a subject for future research.

The second extracellular loop of PLP contains two disulphide bonds, located between Cys183-Cys227 and Cys200-Cys227, respectively. The membrane-proximal

disulphide bond (Cys183-Cys227) is fundamental to the proper cell surface expression of PLP, and it is involved in Pelizaeus–Merzbacher disease [71]. The PLPextra2 peptide contains only two Cys residues (Cys183 and 200) and cannot form these disulphide bonds. However, all membrane-mimicking conditions induced an α -helical folding of the PLPextra2 peptide (Fig. 6d, e and Additional file 5: Table S2). Dhaunchak and Nave [71] also reported that the cell surface expression of PLP can be rescued if the cysteines in the second extracellular loop are mutated, indicating that the disulphide bonds are not crucial for the normal folding of the second extracellular loop.

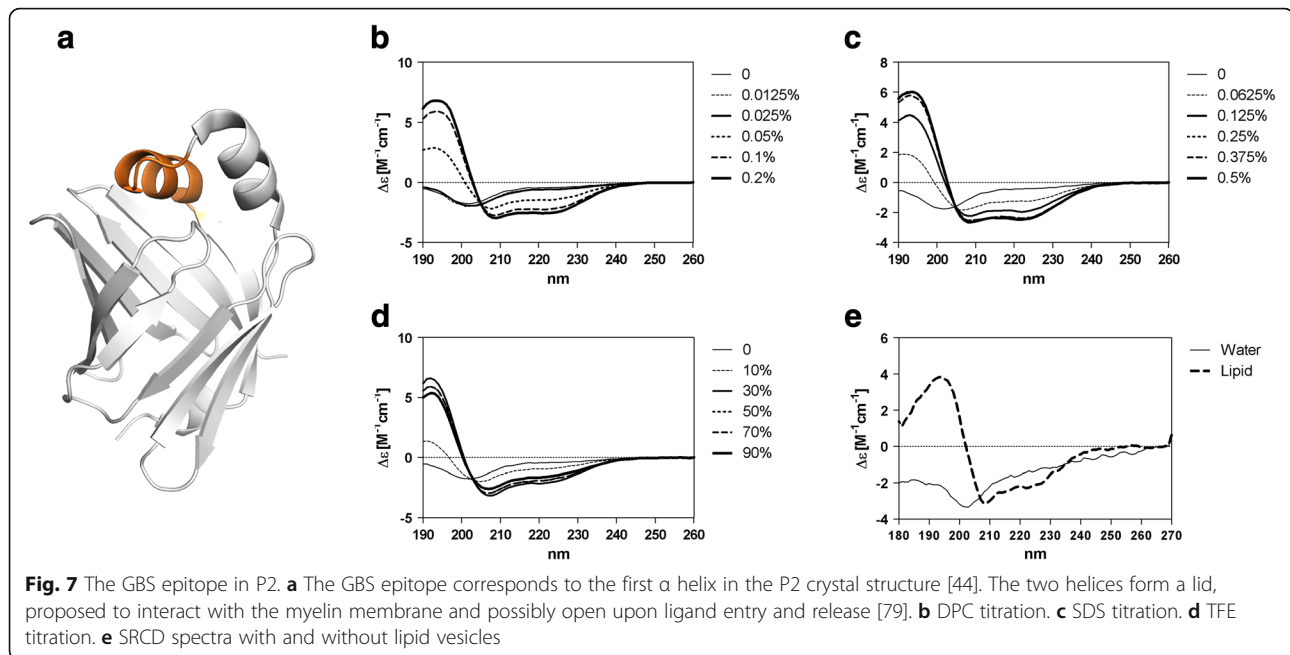
The deconvolution of spectral data suggested 30–40% helical content in membrane vesicles for PLPextra2 and PLPintra1, which is consistent with the *de novo* predicted situation with partially folded and disordered state (Table 1). Although the change in spectrum indicated increased α -helical nature for PLPintra2, the calculated content of α helices was modest. This may be an underestimate due to putative aggregation, which is discussed below.

Membrane-induced folding of the GBS epitope from human peripheral nerve protein P2

P2 is one of the structurally best-characterized myelin proteins, being highly expressed in PNS myelin [72, 73]. It is a proposed target of autoimmune reactions in GBS, and P2 peptides can be used to induce EAN, the mouse model of human GBS [74, 75]. P2 is able to stack lipid bilayers together in an ordered manner in vitro [41, 76, 77], and the P2 knockout mouse has changes in myelin lipid composition and motor nerve conduction velocity during periods of active myelination [73].

The peptide studied here corresponds to the GBS epitope characterized earlier [78], and in the crystal structure, it comprises the first α helix of the lid of the 10-stranded β -barrel structure [44] (Fig. 7a). It is likely that the helical lid is centrally involved in membrane interactions during membrane stacking [79].

The P2gbs peptide showed helical folding at DPC and SDS concentrations above the CMC, and strong helical folding was present at 30% TFE (Fig. 7b–d). With lipid vesicles, a helical spectrum was observed in SRCD experiments, further indicating induction of α -helical folding of P2gbs in membrane-like conditions (Fig. 7e). While these results are not unexpected in light of *de novo* predictions (Table 1, Additional file 3: Figure S2) and the fact that the sequence is helical in the crystal structure and predicted to contact the membrane, they provide striking similarity to the CNS antigen peptides described above. Similar molecular mechanisms and principles could, thus, be involved in demyelination-related autoimmunity in the CNS and PNS.



Turbidimetry shows peptide-induced vesicle agglutination

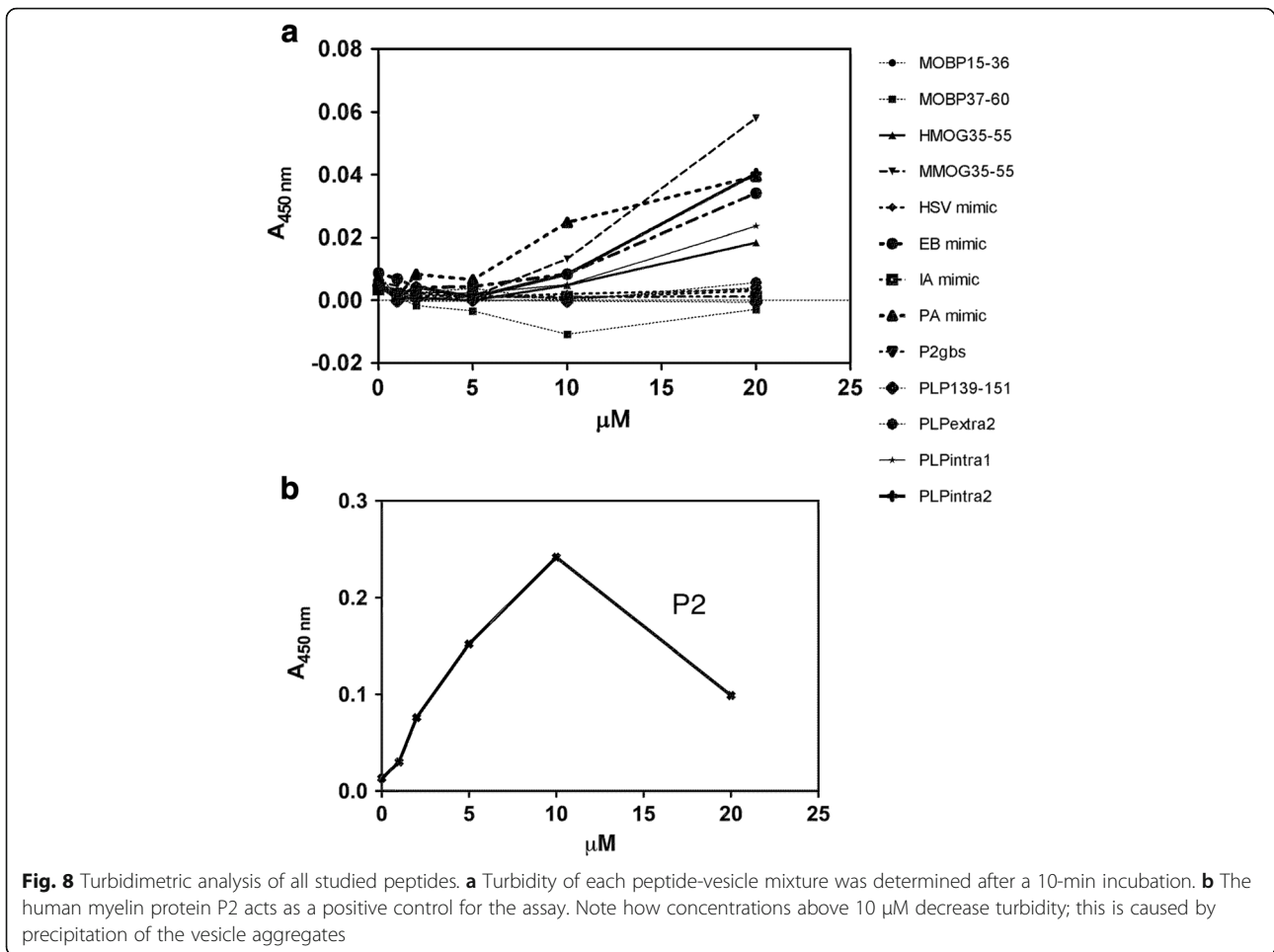
In SRCD, one generally can use shorter pathlengths, and hence, much higher sample concentrations than in conventional CD, which has the problem of light intensity falling off at lower wavelengths. During SRCD experiments, peptide-induced aggregation was observed when lipid vesicles were added into solutions of high (0.5 mg/ml) concentrations of peptides. This was most severe for the EB-mimic, MOG35-55, and PLPintra2 peptides (data not shown). The problem could be circumvented by using lower peptide concentration (0.25 mg/ml) with a high (1:100) peptide:lipid ratio in SRCD experiments; this observation also triggered us to investigate the agglutination potency of the studied peptides more closely.

The monitoring of lipid vesicle aggregation was done by measuring absorption (turbidity) at 450 nm after mixing vesicles with increasing concentrations of peptides (Fig. 8a). Although the peptide-induced aggregation was modest compared to protein P2-induced agglutination (Fig. 8b), reproducible vesicle agglutination was detected for the EB-mimic, hMOG35-55, mMOG35-55, and PLPintra2 peptides, and occasionally also for IA-mimic and PA-mimic (Fig. 8 and data not shown). The observation of turbidity could indicate a propensity of the peptide to stack membrane bilayers together into multilayers (mimicking myelin); in the least, it is a sign of altered surface properties of the lipid membrane, promoting aggregation. Hence, we consider turbidity a functional assay for myelin membrane-stacking proteins/peptides. Having this property not only in myelin protein

peptides, but also in some of the molecular mimics, further indicates common properties for the studied encephalitogenic peptides.

Discussion

Despite huge efforts in the past decades, MS has so far evaded the deciphering of its pathogenesis. We do know that in rodent EAE models, an active immunization with myelin antigens, or a passive adoptive transfer of myelin-specific autoreactive Th1/Th17 CD4 lymphocytes, phenocopies many features of MS [80–82]. There exists also understanding that the genetic susceptibility *via* the HLA-DR15 haplotype and certain environmental factors, such as infection by herpes viruses, especially by Epstein-Barr virus, are significant risk factors for MS [83]. However, we do not know whether induction of autoimmunity in MS takes place primarily in the periphery (like in EAE) or in the CNS. In the early stages of MS, the presence of infiltrated T lymphocytes predominates, while in later stages, CNS inflammation becomes self-sustainable, and the number of B cells in lesions increases [2]. However, we do not know the relative importance of CD4 and CD8 T cells or the exact roles of B cells and antibodies. Finally, although the structural similarity between the MHC II-bound MBP-derived peptides and their molecular mimics, like those from EBV polymerase, can explain how the mimic-specific TCRs in CD4 T cells can recognize self-antigens [84, 85], we lack structures of MBP/mimic-bound MHC I complexes. The latter are important, as cytotoxic CD8 T cells can directly attack the oligodendrocytes, which present peptides to them [86]. Neither do we know whether the



autoantibodies, which recognize folded epitopes like those in MOG, may be induced by mimicking antigens.

MBP is the best-characterized myelin protein, both at the level of the full-length protein and as peptide segments. Much of the early research was carried out using MBP purified from tissue [20–25, 27], and more recently, recombinant MBP has also been intensively studied [29, 30, 48, 49]. According to the current view, MBP is intrinsically disordered in solution, but folds into short helical segments upon membrane binding [87]. The structure of MBP on membranes can be further affected by the lipid composition. Here, we did not assess the effects of different lipid compositions on peptide folding, but earlier research has shown that the helical structure of the autoimmune epitope of MBP does slightly change in different lipid membrane compositions [45, 88]. This is of interest because demyelination is linked to changes in lipid composition, and these changes may in turn alter the conformation, localization, and properties of MBP [89]. Furthermore, the epitope conformation may be affected by its presence in either a peptide or a full-length MBP protein [46, 47]. For obtaining detailed local

conformational information, we chose here to focus on short peptide segments. Even for the longest peptides, we believe that the membrane-binding segments will be short helices, as predicted *de novo* (Additional file 3: Figure S2).

How do our observations of the common membrane-induced folding of MBP mimics, and most other peptides studied here, fit into this big picture? It is possible that the propensity to adopt α -helical structure in the experiment merely illustrates similar biophysical properties. However, it is also possible that these peptides do adopt a helical structure *in vivo*, with relevance to the generation of autoimmunity *per se*. Two speculative hypotheses can be presented.

Firstly, the relatively low affinity and non-standard geometry in the binding of TCRs to MHC II-peptide complexes can explain the escape of self-reactive T cells from negative selection [90]. In the known MHC II-MBP/mimic structures, the peptide binds MHC in an extended conformation [84, 85]. Whether the peptide propensity to fold affects rescue from negative selection is not known. It can be speculated that during the

generation of T cell tolerance, the amount of MHC-loadable non-folded self-peptides is low, but may become significant after inflammatory insult. Interestingly, the presence of autoantigenic MBP peptides in the spectrum of proteasomal products increases after MBP immunization [86]. Secondly, it is possible that mimic-specific, such as Epstein-Barr virus-infected and CNS-infiltrated, memory B cells [91] may have affinity against myelin neoepitopes that are exposed in degenerated/insulted myelin.

Neither scenario above suggests that membrane-induced folding universally leads to auto-immunogenicity, but it could be relevant in rare cases where both 1) the environment-induced conformational changes of myelin proteins can lead to changes in processing and/or peptide loading to affect negative selection of T cells or creation of neoepitopes for B cells and 2) the mimicking agent is present at the critical time. It is obvious that many hydrophobic self-sequences are strongly immunogenic [92, 93], but the corresponding T cells go to apoptosis in the thymus. An animal model with genetic or external exposure of myelin proteins/peptides, which do not significantly change the structure of the host protein, but alter membrane-induced conformational changes, might be useful to test these hypotheses.

Conclusions

The understanding of myelin autoimmunity at the molecular level is poor, also taking into account the fact that myelin protein epitopes in general are buried within the myelin sheath, a tightly packed multilayered membrane. On the other hand, these epitopes are hidden from the immune system in a healthy myelin environment, and might only be exposed in case of prior insult to the myelin membrane. All our experiments point towards highly similar properties for all the studied peptides. In this respect, it is of interest to note the possible potential of myelin protein-derived peptides in MS therapy; these include both cyclic and mutated variants of myelin antigen peptides, especially MBP85-99 [59, 94–98]; the design of such peptides could also take into account our observations on similar overall structural and membrane interaction properties of peptides with limited sequence similarity *per se*. While the link between membrane binding, membrane-induced folding, and autoimmune response is unclear, molecular mimicry possibly taking place in autoimmune demyelination may be based on 3D similarity of host and pathogen epitopes. In the case of MBP85-99 mimics, for example, while the peptides fold into amphipathic helices on lipid membranes, the extended conformation bound to the MHC shows the conserved residues being presented outwards, being important for immune response.

Our results give new insights into myelin protein-membrane interactions within short segments of the protein; the situation in the case of full-length proteins is of course more complex. For protein-peptide interactions, both electrostatic interactions and conformational flexibility are important factors in recognition and binding [99]. In the case of flexible proteins, such as MBP, a multitude of conformations are present [29, 87], and antibody binding might reflect a fortuitous capture of specific conformations that become more rigid. The conformations of MBP peptides on the membrane surface and when bound to MHC may reflect such a phenomenon. Similar properties between different peptide epitopes may provide means to multifaceted, but shared, recognition mechanisms, which in turn may relate to disease etiology. It is clear that more research in *in vivo* systems will be required to gain further answers to these questions in the case of autoimmune demyelination.

Additional files

Additional file 1: Table S1. Details of peptide properties. (PDF 60 kb)

Additional file 2: Figure S1. Helical wheel predictions of all peptides from this study. (PDF 1382 kb)

Additional file 3: Figure S2. PEP-FOLD model predictions for the studied peptides. (PDF 398 kb)

Additional file 4: Figure S3. CD spectra for a negative control peptide from the *P. falciparum* formin display no folding under membrane-mimicking conditions. The peptide sequence is KKIPAPPPFLKKK. (TIF 25 kb)

Additional file 5: Table S2. Results from CD spectrum deconvolution of all 290 samples, with two different algorithms. (PDF 66 kb)

Abbreviations

CD: Circular dichroism; CMC: Critical micellar concentration; CNS: Central nervous system; DMPC: Dimyristoyl phosphatidylcholine; DMPG: Dimyristoyl phosphatidylglycerol; DPC: n-Dodecyl phosphocholine; EAE: Experimental autoimmune encephalomyelitis; EAN: Experimental autoimmune neuritis; GBS: Guillain-Barré syndrome; MBP: Myelin basic protein; MHC: Major histocompatibility complex; MOBP: Myelin-associated oligodendrocytic basic protein; MOG: Myelin-oligodendrocyte glycoprotein; MS: Multiple sclerosis; OCD: Oriented CD; PLP: Proteolipid protein; PNS: Peripheral nervous system; SDS: Sodium dodecyl sulfate; SRCD: Synchrotron radiation CD; TCR: T cell receptor; TFE: Trifluoroethanol

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Availability of data and materials

All data generated or analysed during this study are included in this published article and its Additional files. Raw data files are available from the corresponding author on reasonable request.

Authors' contributions

JT planned and carried out experiments, analysed data, and was a major contributor in writing the manuscript. AR planned and carried out experiments, optimized methodologies, and participated in writing the manuscript. SR analysed data and participated in writing the manuscript. PK obtained funding for the study, planned experiments and analysed data, and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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