Stability profile of Myelin Oligodendrocyte Glycoprotein Peptide in solid and liquid state

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Abstract

Multiple Sclerosis (MS) is a slowly progressive, immunologically mediated disease of the central nervous system, characterized by inflammation and demyelination of white matter in the brain and spinal cord [1,2]. We have previously presented that the immunodominant epitope of myelin oligodendrocyte glycoprotein (MOG_{35-55}) conjugated to mannan polysaccharide ameliorates the clinical symptoms and neuropathology in experimental autoimmune encephalomyelitis (EAE) animal model for MS; therefore represents a promising candidate for peptide-specific immunotherapy [3]. The purpose of the present study was to examine the stability behavior of MOG peptide under the influence of neutral and alkaline conditions, the latter used in the production process of MOG_{35-55} peptide – conjugate.

The results demonstrated that under neutral conditions no conversion was observed; however, upon alkaline conditions deamidation of Asn in position 53 of the peptide sequence occurs, converting Asn to Asp [4].

The functional efficacy of deaminated by-product was evaluated using the EAE model. Interestingly, the deaminated OM-MOG containing the Asp53 showed similar strong protective effects against EAE compared to the native OM-MOG. These results indicate that the presence of Asp53 will not negatively impact the immunomodulatory properties of OM-MOG conjugate prepared by conventional synthetic procedures.

Methods

Synthesis of target peptides and coupling into mannan polysaccharide

The following peptides were synthesized via Fmoc/tBu methodology using 2-chlorotrityl chloride resin (CLTR-Cl).

Peptide	Peptide Sequence	Abbreviation
Pep-1	H-Lys-Gly-Lys-Gly-Lys-Gly-Lys-Gly-Lys-Gly-Met[35]-Glu-Val-Gly-Trp-Tyr-Arg-Pro-Pro-Phe-Ser-Arg-Val-Val-His-Leu-Tyr-Arg-**Asn[53]**-Gly-Lys[55]-OH	$(KG)_5MOG_{35-55}$
Pep-2	H-Lys-Gly-Lys-Gly-Lys-Gly-Lys-Gly-Lys-Gly-Met[35]-Glu-Val-Gly-Trp-Tyr-Arg-Pro-Pro-Phe-Ser-Arg-Val-Val-His-Leu-Tyr-Arg-**Asp[53]**-Gly-Lys[55]-OH	$(KG)_5MOG_{35-55}(Asp^{53})$
Pep-3	H-Lys-Gly-Lys-Gly-Lys-Gly-Lys-Gly-Lys-Gly-Met[35]-Glu-Val-Gly-Trp-Tyr-Arg-Pro-Pro-Phe-Ser-Arg-Val-Val-His-Leu-Tyr-Arg-**isoAsp[53]**-Gly-Lys[55]-OH	$(KG)_5MOG_{35-55}(isoAsp^{53})$
Pep-4	H-Lys-Gly-Lys-Gly-Lys-Gly-Lys-Gly-Lys-Gly-Met[35]-Glu-Val-Gly-Trp-Tyr-Arg-**Ser[42]**-Pro-Phe-Ser-Arg-Val-Val-His-Leu-Tyr-Arg-**Asn[53]**-Gly-Lys[55]-OH	$(KG)_5MOG_{35-55}$ (Ser^{42})
Pep-5	H-Lys-Gly-Lys-Gly-Lys-Gly-Lys-Gly-Lys-Gly-Met[35]-Glu-Val-Gly-Trp-Tyr-Arg-**Ser[42]**-Pro-Phe-Ser-Arg-Val-Val-His-Leu-Tyr-Arg-**Asp[53]**-Gly-Lys[55]-OH	$(KG)_5MOG_{35-55}$ $(Ser^{42}$, $Asp^{53})$
Con-Pep4	Oxidized Mannan — KGKGKGKGKG–$MOG_{35-55}(Ser^{42})$	OM-$(KG)_5MOG_{35-55}$ (Ser^{42})
Con-Pep5	Oxidized Mannan — KGKGKGKGKG–$MOG_{35-55}(Ser^{42}$, $Asp^{53})$	OM-$(KG)_5MOG_{35-55}$ $(Ser^{42}$, $Asp^{53})$

The synthesized peptides were conjugated into mannan (poly-mannose from *Saccharomyces Cerevisiae*), in its oxidized form using the following procedure. Mannan was oxidized to poly-aldehyde using sodium periodate and purified by size exclusion chromatography (Sephadex G-25 Medium column). The purified oxidized mannan was mixed with the peptides and incubated at room temperature for 48 hours. The completion of the conjugation reaction was confirmed by Tricine SDS-PAGE.

Evaluation of the stability of peptides in solid and liquid form during manufacturing of mannan-peptide conjugates via HPLC & NMR studies

Stability is a major concern in manufacturing. The present study involves the investigation of deamidated products during conjugation reaction while manufacturing mannan peptide conjugates in basic conditions. The conjugation reaction of MOG peptides with oxidized mannan occurs in sodium bicarbonate buffer, pH 9.0. The synthesized peptides were stored at -18°C ± 2°C and analyzed by HPLC. Furthermore, Pep-1 and Pep-4 (MOG human & rat sequence) were diluted to sodium bicarbonate buffer solution, pH 9.0 and analyzed by HPLC at t=0, 24 & 48h after dilution. The results demonstrated that in both sequences, an unknown peak was formed, immediately after dilution and was stabilized at about 16h (Figure 1). For further investigation and confirming the theoretical hypothesis that the revealed unidentified peaks were due to the formation of deamidated peptides either containing Asp or Iso-Asp in the position of Asn, Pep-2, Pep-3 and Pep-5 were synthesized and evaluated via HPLC and NMR studies.

Results and Discussion

The chemical behavior of MOG peptides demonstrated that they remain stable when stored in solid form at -18°C ± 2°C and also when dissolved in purified water for at least 48h.

With respect to the unknown peaks revealed in the chromatograms, when peptides are dissolved in sodium bicarbonate buffer, pH 9.0, results demonstrated that the peptides undergo deamidation and Asn at position 53 is converted to Asp.

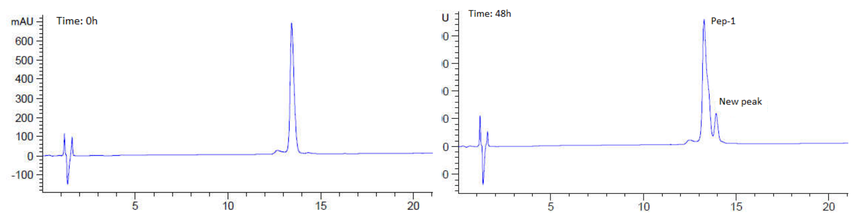

Figure 1: RP-HPLC chromatogram of Pep-1 at 214.4 nm at time zero & 48h after dilution in sodium bicarbonate buffer.

The NMR studies confirmed the above findings.

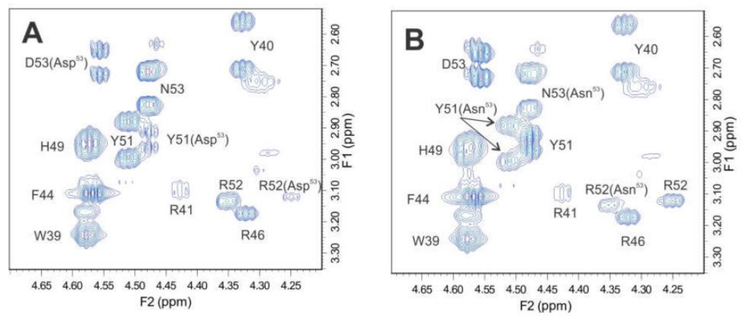

Figure 2: Expanded regions of TOCSY spectra of: **(A)** Pep-1 and **(B)** isolated new peak after semipreparative-HPLC purification.

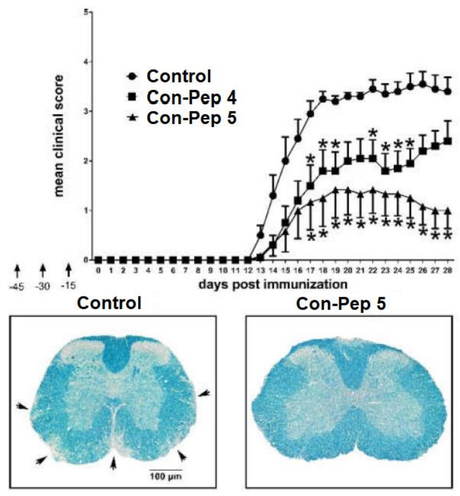

Figure 3: Administration of Con-Pep 4 and Con-Pep 5, conjugated to mannan in its oxidized form, in a prophylactic (vaccination) protocol attenuates the development of MOG-induced experimental autoimmune encephalomyelitis (EAE) in C57BL/6 mice.

Top: Mean clinical scores of MOG-EAE in groups of mice vaccinated intradermally (i.d.) with dilute soluble Con-Pep 4, Con-Pep 5 or sodium bicarbonate buffer pH 9.0 (disease control) at the indicated time points (arrows), prior to the induction of EAE by immunization with MOG/CFA/PTx (*n* = 10 for Con-Pep 4 and control groups, *n* = 6 for Con-Pep 5 group).

Bottom: MOG-EAE vehicle control mice induces hallmark demyelinating lesions, as seen by Luxol Fast blue staining of spinal cord sections taken from mice sacrificed on day 28 postimmunization for EAE (left panel, arrows). Vaccination with either Con-Pep 4 (data not shown) or Con-Pep 5 prevented the development of spinal cord demyelination (right panel). Representative sections from three animals per group are shown. Statistical significance after comparisons between Con-Pep 4 and 5 treated with control groups of mice by 2-way ANOVA followed by Bonferroni posthoc tests is shown (* $p \leq 0.05$).

References

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