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Analysis of Mannan (Polymannose)-Peptide Conjugate by Competitive ELISA

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Abstract

Multiple sclerosis (MS) is an autoimmune disease whereby the myelin of the central nervous system (CNS) is destroyed, leading to paralysis and serious health problems [1]. The main proteins of the membrane sheath of myelin are: i) myelin basic protein (MBP); ii) myelin oligodendrocyte glycoprotein (MOG), iii) myelin-associated glycoprotein (MAG) and iv) proteolipid protein (PLP) [2,3]. Immunodominant epitopes of myelin proteins are involved in pathogenesis of MS. Thus, the 35-55 sequence of MOG protein is an autoantigen of MS and induces *in vivo* Experimental Autoimmune Encephalomyelitis (EAE-animal model of MS) [3]. Moreover, the conjugate of this epitope with mannan (polysaccharide) was found to inhibit the EAE symptoms and could be a new and promising approach for MS treatment [4,5]. In the present study, a competitive ELISA was developed using the MOG_{36-55} peptide for the coating of polystyrene microtiter plates, polyclonal antibodies produced in rabbits after immunization with this peptide, and the mannan-MOG conjugates as competitors. The first approach was to test the stability of mannan-MOG conjugates during storage conditions and their potency after modifications at the peptide sequence that may occur spontaneously. The developed methodology is possible to be used in further analysis of mannan – peptide conjugate in combination with *in vivo* experiments

Methods

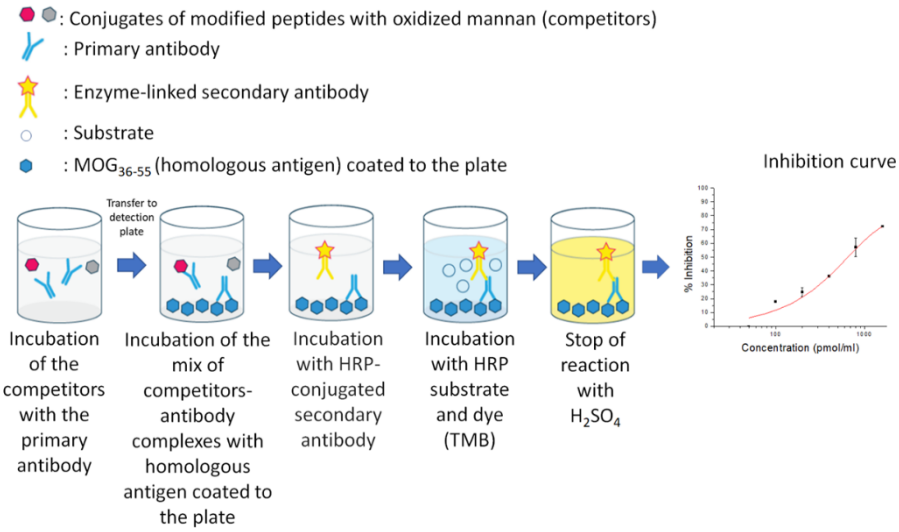
Synthesis and coupling of the immunodominant epitopes to mannan

The studied peptides were synthesized by Fmoc/tBu methodology using 2-chlorotrityl chloride resin (CLTR-Cl). The synthesized peptides were purified and identified by semi preparative HPLC and mass spectrometry (ESI-MS) respectively with a purity yield more than 98%. Mannan (poly-mannose from Saccharomyces Cerevisiae), was oxidized to poly-aldehyde using sodium periodate ($NaIO_4$) and purified by size exclusion chromatography (Sephadex G-25 Medium column). The purified oxidized form of mannan was mixed with the peptides and incubated at room temperature for 48 hours. The conjugation of aldehydes, after the oxidation of mannan, with the amines of the Lys residues {$[KG]_5$ linker} of the peptides was achieved via base Schiff formation, while the completion of the conjugation reaction was confirmed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) [5].

Evaluation of the stability of mannan-peptide conjugates with ELISA

A competitive ELISA was developed using $[KG]_5MOG_{35-55}$ peptide with the sequence H-Lys-Gly-Lys-Gly-Lys-Gly-Lys-Gly-Lys-Gly-Met^{35}-Glu^{36}-Val^{37}-Gly^{38}-Trp^{39}-Tyr^{40}-Arg^{41}-Pro^{42}-Pro^{43}-Phe^{44}-Ser^{45}-Arg^{46}-Val^{47}-Val^{48}-His^{49}-Leu^{50}-Tyr^{51}-Arg^{52}-Asn^{53}-Gly^{54}-Lys^{55}-OH. Affinity purified polyclonal antibodies against peptide MOG_{36-55} (sequence 36-55 of $[KG]_5MOG_{35-55}$) were produced in rabbits via immunization process by Davids Biotechnologies GMBH (Germany), while the epitope recognized by the antibodies is located into sequence 38-49.

The developed ELISA includes the following procedure:



Several modifications of peptide sequence may occur during the peptide synthesis and storage. For the purpose of this study, we have also synthesized and analyzed the oxidized Met in MOG_{35-55} epitope conjugated to mannan. Thus, the following conjugates were studied:

i) Man^{ox} -$[KG]_5MOG_{35-55}$
ii) Man^{ox} - $[KG]_5MOG_{35-55}(Met(O)^{35})$
iii) Man^{ox} - $[KG]_5MOG_{35-55}$, stored at -20°C for at least 36 months

The ability of anti-MOG_{36-55} antibody to react with oxidized mannan (cross-reactivity) was further examined, to investigate the detection of any false-positive result. The results demonstrated that the antibody does not recognize the oxidized mannan.

The Coefficient of Variation (CV) was calculated to be 14.56% and 4.45% for Inter and Intra assay, respectively, so the competitive ELISA is repeatable and accurate and can be used to evaluate the stability of the conjugate.

Results

The study of the conjugates by competitive ELISA, showed that possible alterations of MOG_{35-55} epitope (chemical and/or conformational changes) affect the recognition from the specific anti-MOG_{36-55} antibody (Figure 1).

- The conjugate of mannan with wild type peptide led to a range of inhibitions 0-80% for the studied concentrations (■, red line).
- The Met oxidation is a common mutation that is easily achieved during the deprotection step of peptides. This mutation leads in enhanced reaction for all the examined concentrations of the conjugate Manox-$[KG]_5MOG_{35-55}(Met(O)^{35})$ with anti-MOG_{36-55} antibody and subsequently to a reduced range of inhibition 35-70% (►, purple line).
- The examined conjugate when stored at -20°C, led to a lower binding affinity between the anti-MOG_{36-55} antibody and the conjugate (at lower concentrations) and subsequently to a reduced range of inhibition 25-55% (□, green line).

Except for the chemical modifications, the reduced range of inhibition of studied peptides conjugated with mannan in comparison with the wild type may occurs due to conformational changes of conjugates that reduce the recognition of the peptide by the antibody.

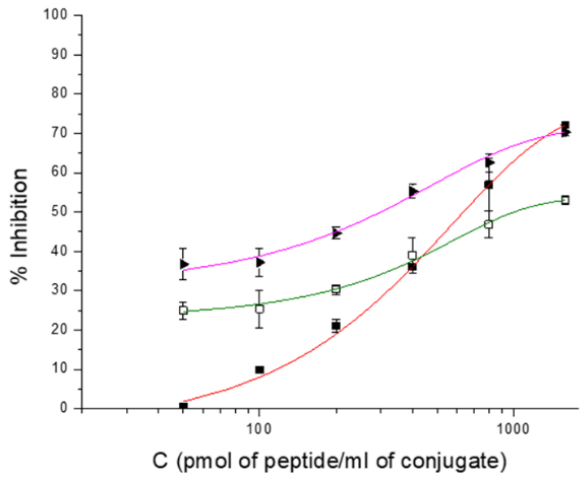


Fig. 1. Inhibition curves of conjugates of oxidized mannan with the modified peptide analogues of MOG_{35-55} using polyclonal antibodies against MOG_{36-55} and increased concentration (50-1600pmol peptide/ml) of: Man^{ox} -$[KG]_5MOG_{35-55}$ (■, red line); Manox - $[KG]_5MOG_{35-55}$, stored at -20°C (□, green line); Manox - $[KG]_5MOG_{35-55}(Met(O)^{35})$ (►, purple line). Each data point represents the mean value of a triplicate measurement ± SD.

The study of the conjugates by competitive ELISA, showed that possible alterations of MOG_{35-55} epitope (chemical and/or conformational changes) affect the recognition from the specific anti-MOG_{36-55} antibody (Figure 1).

Conclusions

The mannan-$[KG]_5MOG_{35-55}$ conjugate is of great interest in the fight against MS and is a promising candidate for clinical studies [4,6]. The developed methodology is sensitive and reliable and could be used to control mutations of peptide sequence during storage.

Acknowledgments

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