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Analysis of Mannan (polymannose) Conjugated with 35-55 Immunodominant Epitope of Myelin Oligodendrocyte Glycoprotein for the Treatment of Multiple Sclerosis

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Introduction

Multiple sclerosis (MS) is a prevalent autoimmune disease characterised by the destruction of myelin, leading to paralysis and severe health complications. The immunodominant epitope 35-55 of myelin oligodendrocyte glycoprotein (MOG_{35-55}) is a crucial autoantigen implicated in the progression of MS. Numerous studies have focused on the use of mannan polysaccharide (derived from Saccharomyces cerevisiae) conjugated with the MOG_{35-55} epitope to induce antigen-specific immune tolerance against the clinical symptoms of chronic experimental autoimmune encephalomyelitis (EAE), an animal model of MS [1,2]. In this context, we present a promising approach to MS immunotherapy where the MOG_{35-55} peptide is conjugated to mannan *via* Schiff base formation between the aldehydes of oxidised mannan and the amino groups of the $(LysGly)_5$ bridge located at the *N*-terminus of the peptide [3]. To confirm the integrity of the conjugation reaction and investigate any potential alterations in the peptide throughout the different stages of mannan-MOG_{35-55} conjugate production, a competitive enzyme-linked immunosorbent assay (ELISA) was developed. This assay was used in combination with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and a colorimetric assay. **These mannan-MOG_{35-55} conjugates are currently undergoing a Phase I clinical trial conducted by a Greek pharmaceutical company.**

Experimental

Synthesis and conjugation of MOG_{35-55} to mannan

The [$(LysGly)_5MOG_{35-55}$] was synthesised on CLTR-Cl resin using the Fmoc/tBu methodology. Purification and identification of the peptide were achieved by semi-preparative RP-HPLC and ESI-MS, respectively. Mannan (poly-mannose from Saccharomyces cerevisiae) was oxidised to poly-aldehyde using sodium periodate ($NaIO_4$) and purified by size exclusion chromatography using a Sephadex G-25 Medium column. The [$(LysGly)_5MOG_{35-55}$] peptide was then mixed with oxidised mannan and incubated for 48 h at room temperature. Conjugation was achieved through the formation of a Schiff base between the aldehydes of the oxidised mannan and the amines of Lys residues in the $(LysGly)_5$ linker. The completeness of the reaction was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

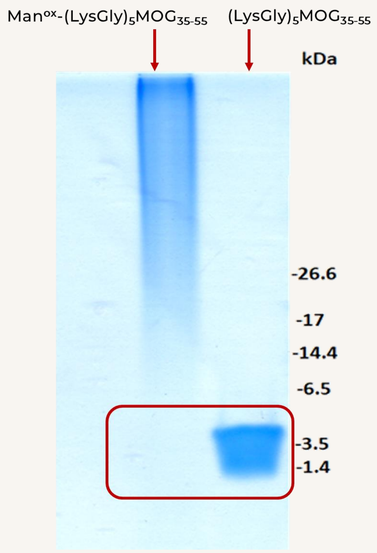


Fig.1: Confirmation of the conjugation of oxidised mannan with $(LysGly)_5MOG_{35-55}$ was achieved by SDS-PAGE in a 16.5% acrylamide gel and Coomassie Brilliant Blue staining. The conjugate of oxidised mannan with the peptide analogue $(LysGly)_5MOG_{35-55}$ appears as a diffuse band ranging from 20-200 kDa due to the variability in the number of mannose molecules in the main and side chains of the polysaccharide, leading to differentiation in peptide-mannan conjugation. The band with a molecular weight of 3.0-3.5 kDa corresponds to the peptide $(LysGly)_5MOG_{35-55}$. **In the lane containing the conjugate, no band corresponding to the free peptide (indicated by the red square) is observed, indicating that the conjugation is successful.**

Stability study of the conjugate stored at -20 °C ± 2 °C

A competitive ELISA was developed to detect the MOG_{35-55} peptide, even when conjugated with mannan polysaccharide. This method can identify alterations in the MOG_{35-55} epitope that may occur during storage at -20 °C ± 2 °C for at least three years.

Concentration of peptide in the conjugate (pmol/mL)	Log (C)	Average %Inhibition	Average %Inhibition
1600	3.20	54.96	72.14
800	2.90	49.61	57.04
400	2.60	44.84	36.11
200	2.30	33.68	24.60
100	2.00	30.69	17.91
50	1.70	24.64	0.07

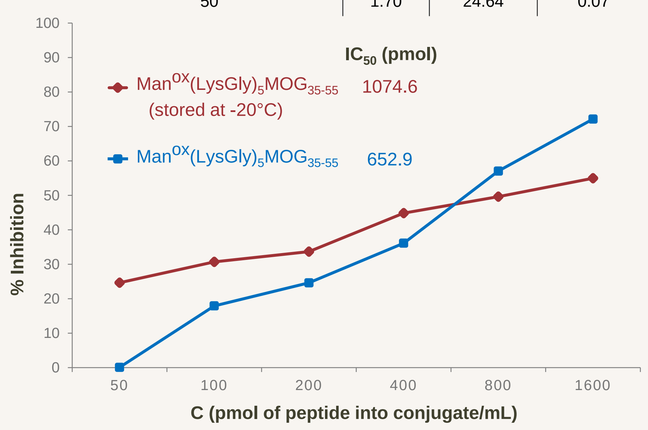


Fig.2: Inhibition curves for conjugate Man^{ox}-$(LysGly)_5MOG_{35-55}$ using polyclonal antibodies against MOG_{36-55} at six concentration levels of the peptide (50–1600 pmol/mL). Each data point represents the mean value of a triplicate measurement ± SD**. The stored conjugate showed increased recognition at low concentrations (50–400 pmol/mL) or reduced recognition at high concentrations (800–1600 pmol/mL) by the antibody. The reduced range of inhibition percentage indicates decreased recognition of the epitope by the antibody. This is likely due to potential alterations of the peptide or conformational changes that hinder the antibody's ability to recognize the epitope.**

Study of the oxidative conditions and parameters for mannan oxidation

The oxidation of mannan was studied to optimize the conditions, specifically reaction time and the amount of oxidizing agent, using the reagent 2,4-dinitrophenylhydrazine (DNPH). The absorbance of the resulting yellow-coloured product was measured at 349 nm.

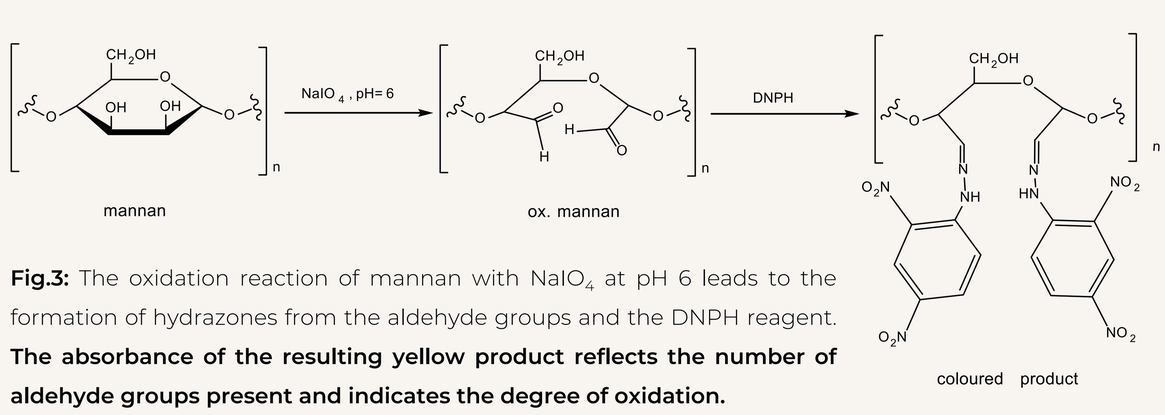


Fig.3: The oxidation reaction of mannan with $NaIO_4$ at pH 6 leads to the formation of hydrazones from the aldehyde groups and the DNPH reagent. **The absorbance of the resulting yellow product reflects the number of aldehyde groups present and indicates the degree of oxidation.**

Oxidizing Agent Monitoring

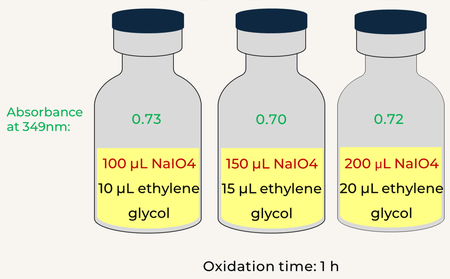
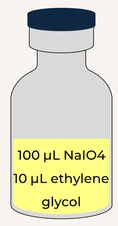


Fig.4: Mannan samples were subjected to an oxidative reaction for 1 h using three different amounts of $NaIO_4$. The **absorbances measured for these samples indicated that all three amounts of the oxidizing agent resulted in the same degree of oxidation.**

Reaction Time Monitoring



Oxidation time	Absorbance at 349nm
0 min	0.48
5 min	0.76
30 min	0.83
1 h	0.78
1.5 h	0.76
2 h	0.75

Fig.5: Oxidation reaction of mannan samples using a constant amount of $NaIO_4$ was conducted over 6 different reaction times. **The absorbances of these samples showed that the reaction was completed within 5 min.**

Conclusions

The Man^{ox}-$(LysGly)_5MOG_{35-55}$ conjugate is of significant interest in the fight against multiple sclerosis (MS) and is currently in phase I clinical trials. The developed methodology is both sensitive and reliable, making it suitable for monitoring peptide mutations of the conjugate during synthesis and storage. Furthermore, these methods could be applied not only to mannan conjugates but also to the analysis and identification of various bioactive glycoproteins.

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