NMR structural elucidation of mannan (polymannose) conjugate with the myelin oligodendrocyte glycoprotein 35-55 epitope (MOG_{35-55})

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Abstract

Multiple sclerosis (MS) is a slowly progressive, chronic inflammatory, autoimmune disease of the Central Nervous System (CNS), characterized by destruction of the myelin sheath leading to paralysis and serious health problems [1-4]. The myelin oligodendrocyte glycoprotein MOG is a main myelin protein and is implicated in the progress of MS [1, 5]. The epitope 35-55 from MOG protein is an autoantigen associated with the pathogenesis of MS and the induction of Experimental Autoimmune Encephalomyelitis (EAE; animal model of MS) in mice [5]. Moreover, the conjugate of 35-55 epitope with mannan polysaccharide was found to inhibit the EAE symptoms and could be a promising candidate for MS treatment [6, 7].

Mannose, mannan polysaccharide, oxidized mannan and the conjugates of immunodominant MOG_{35-55} epitope with-mannan in oxidized form were studied by high field nuclear magnetic resonance (NMR) spectroscopy to explore the structural characteristics of mannan and its conjugate with MOG_{35-55} epitope. This study under progress aims: (a) to detect spatial interactions between MOG_{35-55} epitope and mannan that possibly determine the active conformation of the complex; (b) to identify the stability of the product under storage conditions at room and cold temperatures; (c) to detect the degree of oxidation of mannan polysaccharide.

Methods

Samples for NMR analysis.

The samples used for NMR analysis were mannose, mannan, oxidized mannan and conjugates of oxidized mannan with the immunodominant epitope MOG_{35-55}. Oxidized mannan was synthesized after oxidation of mannan to poly-aldehyde using sodium periodate ($NaIO_4$) and purified by size exclusion chromatography (Sephadex G-25 Medium column) and the conjugates of oxidized mannan with the peptide $[KG]_5MOG_{35-55}$ were synthesized by mixing of the peptide with the oxidized mannan and incubation at room temperature for 48h. The conjugation was achieved by formation of Schiff base between the aldehydes of the oxidized polysaccharide and the free amines of the peptide. The liquid samples of oxidized mannan and conjugates were lyophilized. Two additional conjugates that were stored at +5°C and at -20°C for at least 3 years, were also lyophilized. Afterwards all samples mannose (15.25mg), mannan (17.78mg), oxidized mannan (24.72mg) and conjugates (23.91mg, 25.08mg and 25.0mg for fresh, at -20°C and 5°C respectively) were dissolved in 750μL of D_2O and 0.67mM NaTMSP (internal reference).

NMR analysis.

The experiments were performed, using Agilent Technologies VNMRS 800MHz spectrometer (Bruker Biospin - TCI probe - Four channel AVANCE NEO console). The structural elucidation of samples of mannose, mannan, oxidized mannan and conjugates of oxidized mannan with $[KG]_5MOG_{35-55}$, was achieved by analysis of 1D (**^{1}H ,^{13}C**) and 2D homonuclear and heteronuclear experiments (**2D COSY, 2D ROESY, 2D TOCSY, 2D HSQC and 2D HMBC**). Analytically, parameters such as receiver gain, relaxation delay and number of scans were at 32, 10.0000 and 16 correspondingly.

Acknowledgments

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Results

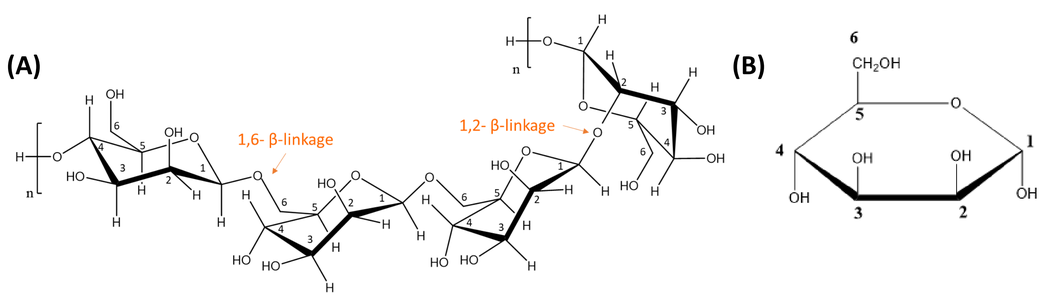

Figure 1: Mannan from *Saccharomyces Cerevisiae* (polymer of D-mannose) **(A)**; D-mannose **(B).**

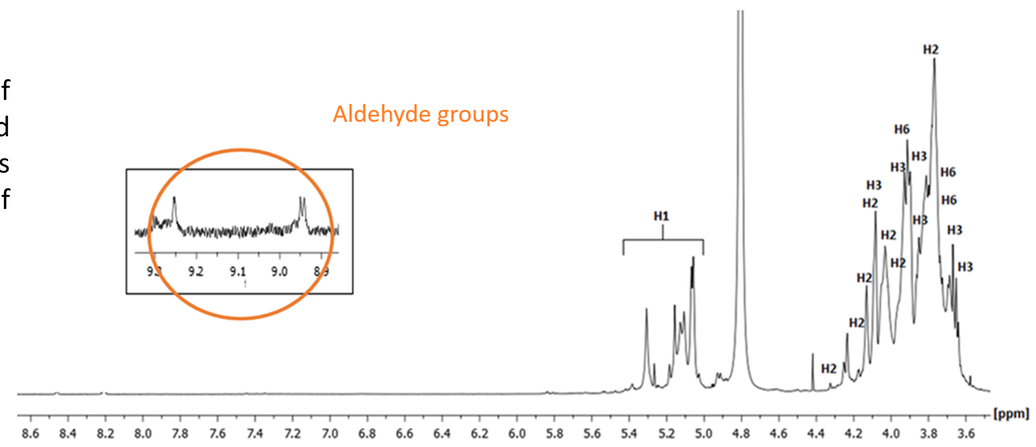

Figure 2: A 800MHz ^{1}H NMR spectrum of oxidized mannan. Peaks at 8.98ppm and 9.25ppm correspond to aldehyde groups which are formed after the oxidation of mannan with $NaIO_4$.

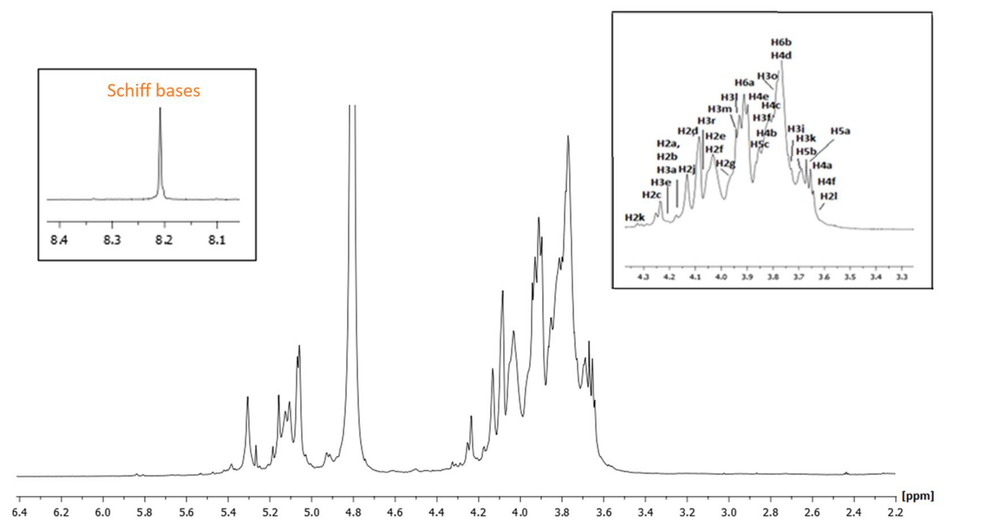

Figure 3: A 800MHz ^{1}H NMR spectrum of conjugate of the oxidized polysaccharide with the peptide $[KG]_5MOG_{35-55}$. Peak at 8.2ppm corresponds to imines (Schiff Bases) which are formed between the produced aldehydes groups of the oxidized mannan and the free amino-groups of the peptide (the free amino-groups of the Lys of the linker $[KG]_5$). The peaks for the aldehyde groups are absorbed after the conjugation reaction. Similar results were obtained for the three different conjugates: i) fresh prepared ii) at least 3 years stored at +5°C and iii) at least 3 years stored at -20°C.

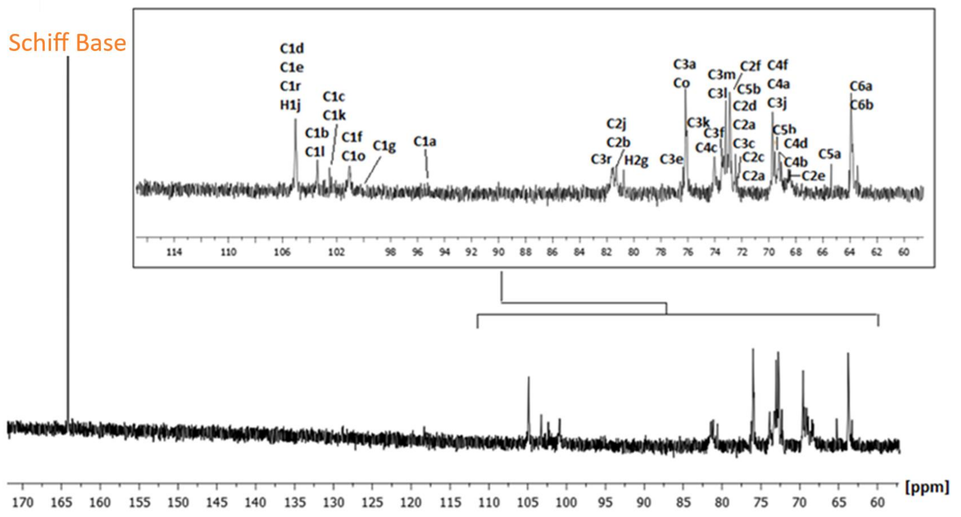

Figure 4: A 800MHz ^{13}C NMR spectrum of conjugate of the oxidized polysaccharide with the peptide $[KG]_5MOG_{35-55}$. Peak at 165ppm corresponds to imines (Schiff Bases). Similar results were obtained for the three different conjugates: i) fresh prepared ii) at least 3 years stored at +5°C and iii) at least 3 years stored at -20°C.

Conclusions

- The oxidation of mannan leads to the emergence of two peaks at the ^{1}H NMR which correspond to the formed aldehydes. These two peaks are not observed after the conjugation reaction of oxidized mannan with the peptide and a new peak appears. This peak corresponds to the Schiff bases which are formed between the amines of the peptide and the aldehydes.
- The conjugate, based on the NMR spectrum, for the three samples (fresh prepared; at least 3 years stored at +5°C; at least 3 years stored at -20°C) is stable under storage conditions.
- The degree of oxidation in the oxidized mannan was found after comparison of the aldehyde peak and one anomeric to be 9.7%.

VIANEX S.A. PHARMACEUTICAL MANUFACTURERS MEMBER OF GIANNAKOPOULOS GROUP

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