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NMR Structural Elucidation of Mannan (Polymannose) Conjugate with the Myelin Oligodendrocyte Glycoprotein 35-55 Epitope (MOG_{35-55})

Areti Gkika[1], Nikoletta Zoupanou[2], Maria-Eleni Androutsou[3], Thomas Mavromoustakos[2], and Theodore Tselios[1]

[1]Department of Chemistry, University of Patras, 26504 Rion Patras, Greece; [2]Department of Chemistry, National and Kapodistrian University of Athens, 10679 Athens, Greece; [3]Vianex S.A., 18th km Athens-Lamia National Road, Nea Erythrea, 14671 Athens, Greece

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 (Figure 1), 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) stability of the product under storage conditions at room and cold temperatures; (c) degree of oxidized mannan achieved in the synthesis.

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 +5ºC and at -20ºC 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 -20ºC and 5ºC 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 (^1H, ^{13}C) and 2D homonuclear and heteronuclear experiments (2D COSY, 2D ROESY, 2D TOCSY, 2D HSQC and 2D HMBC) (Figure 2).

Results

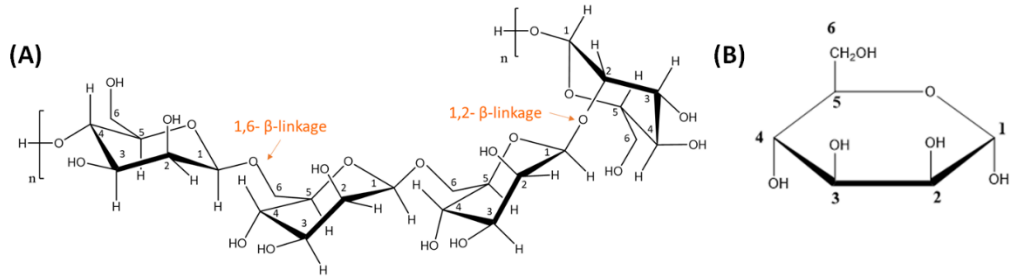
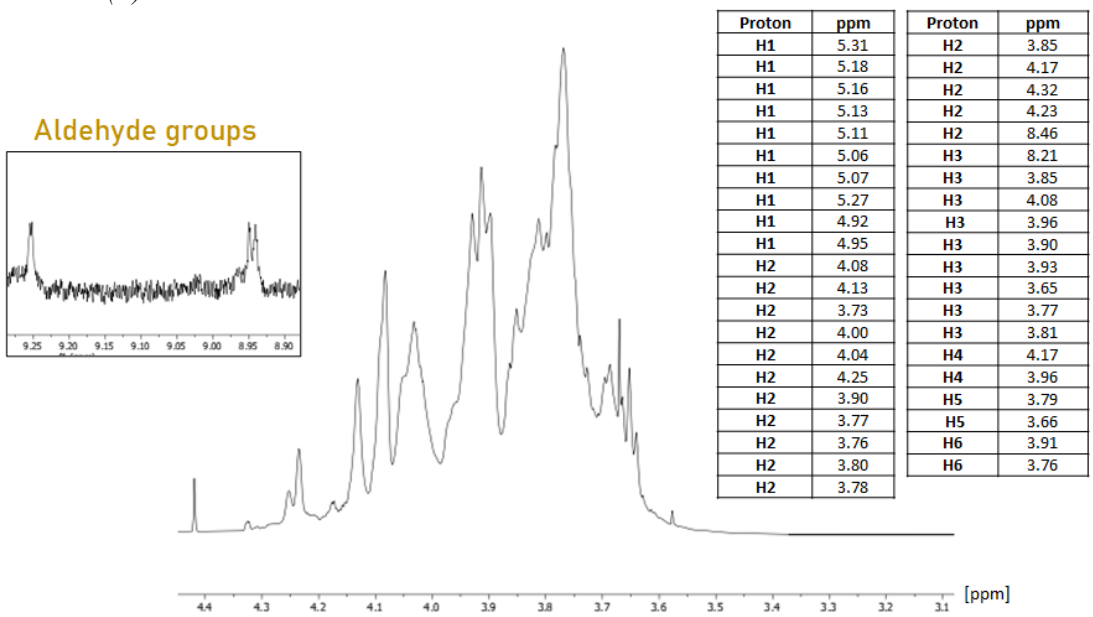


*Fig. 1. Structure of mannan from Saccharomyces Cerevisiae (poly-mannose) (**A**); structure of D-mannose (**B**).*



Proton	ppm
H1	5.31
H1	5.18
H1	5.16
H1	5.13
H1	5.11
H1	5.06
H1	5.07
H1	5.27
H1	4.92
H1	4.95
H2	4.08
H2	4.13
H2	3.73
H2	4.00
H2	4.04
H2	4.25
H2	3.90
H2	3.77
H2	3.76
H2	3.80
H2	3.78

Proton	ppm
H2	3.85
H2	4.17
H2	4.32
H2	4.23
H2	8.46
H3	8.21
H3	3.85
H3	4.08
H3	3.96
H3	3.90
H3	3.93
H3	3.65
H3	3.77
H3	3.81
H4	4.17
H4	3.96
H5	3.79
H5	3.66
H6	3.91
H6	3.76

Fig. 2. Representative part of the 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 $NaIO_4$. The complexity and overlapping of the ^{1}H NMR peaks constituting the spectrum explains the subtle different environment of the protons. The table includes the chemical shifts (ppm) of the protons of oxidized mannan.

Conclusions

i. The oxidation of mannan leads to the emergence of two peaks at the ^{1}H NMR which correspond to the formed dialdehyde. These two peaks are not observed after the conjugation reaction of oxidized mannan with the peptide.
ii. The peaks correspond to the Schiff bases which are formed between the amines of the peptide and the aldehydes and are observed at the range 6.0-8.0ppm. In this range are also observed peaks that correspond to the peptide.
iii. The conjugate, based on the NMR spectrum, for the three samples (fresh prepared; at least 3 years stored at +5ºC; at least 3 years stored at -20ºC) is stable under storage conditions.
iv. The degree of oxidation in the oxidized mannan was found after comparison of the aldehyde peak and one anomeric to be 9.7%.

Acknowledgments

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