Verification of mannan (polymannose)-peptide conjugation by SDS-PAGE

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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] Myelin consists of proteins and lipids while in CNS, myelin basic protein (MBP), proteolipid protein (PLP), myelin oligodendrocyte glycoprotein (MOG) and myelin-associated glycoprotein (MAG) are the main proteins of the membrane sheath of myelin. [2] MOG is an autoantigen associated with the pathogenesis of MS and experimental autoimmune encephalomyelitis (EAE, animal model of MS). The 35-55 epitope of MOG has been implicated in the induction of EAE in mice.[3] Conjugation of this epitope with mannan polysaccharides *via* Schiff base formation has been extensively studied in our laboratory with the conjugates showing potential activity against EAE.[4–6] In the presented study, the conjugation reaction was investigated *via* SDS-PAGE and High-Performance Liquid Chromatography (HPLC) and the unconjugated peptide was quantified during reaction. The optimum time needed for complete conjugation was about six hours (yield more than 95 %). The developed analytical methodology could prove useful assay for the determination and study of conjugation reaction between peptides and polysaccharides.

Aim

The aim of this study was to demonstrate the decrease of [KG]₅MOG₃₅₋₅₅ peptide analogue during conjugation reaction and confirm the totally conjugation with mannan polysaccharide. Samples were taken during conjugation reaction to review the decrease of [KG]₅MOG₃₅₋₅₅ (free peptide) by the time in the conjugation process. Each sample was taken on a time-based period (t = 0, t = 30 min, t = 1h, t = 2h, t = 3h, t = 4h and t = 24h) and was tested *via* SDS PAGE and RP-HPLC. Staining of gels was performed with Coomassie Brilliant Blue.

Methods

Synthesis and coupling of the immuno dominant epitope MOG₃₅₋₅₅ to mannan. The [KG]₅MOG₃₅₋₅₅ peptide was synthesized by Fmoc/tBu methodology using 2-chlorotrityl chloride resin (CLTR-Cl). [6] The synthesized peptide was purified and identified by semi preparative HPLC and mass spectrometry (ESI-MS) respectively with a purity yield more than 95%. Mannan (poly-mannose from *Saccharomyces Cerevisiae*) was oxidized to poly-aldehyde using sodium periodate (NaIO₄) and purified by size exclusion chromatography (Sephadex G-25 Medium column). The purified oxidised mannan (Ox.mannan) was mixed with [KG]₅MOG₃₅₋₅₅ peptide and incubated at room temperature, in dark, for at least 24 hours. The conjugation reaction was achieved between the aldehydes of oxidised mannan and the free amine groups of amino acids in the peptide sequence.

Experimental

Samples which received from the Ox.mannan-[KG]₅MOG₃₅₋₅₅ conjugate reaction, were diluted by adding tricine sample buffer (Figure 1 - Table) and loaded on a tricine gel containing 3.5% stacking gel and 16.5% separating gel. Immediately after ending electrophoresis, gels were stained in 0.1% Coomassie Brilliant Blue G 250 (Figure 1). The free peptide was detected as a band with an apparent molecular weight of 3.0–3.5 kDa therefore the mannan-peptide conjugate appeared as a diffuse smear with an apparent molecular weight of 20–200 kDa. Quantification of unconjugated peptide was achieved by using a standard curve, constructed with known peptide concentrations in gels and the image j program. The detection limit of the method for the peptide quantification with Coomassie Blue stain was 37.5 ng. Furthermore, all samples were analyzed using RP-HPLC to demonstrate the presence of free peptide at time zero during reaction (Figure 2) and the absence of the peptide at the termination of conjugation reaction.

Results

Figure 1: Time point samples of conjugation reaction via Tricine SDS-PAGE. Concentrations and % percentage of unconjugated [KG]₅MOG₃₅₋₅₅ during reaction.

Figure 2: RP-HPLC chromatogram of Ox.mannan-[KG]₅MOG₃₅₋₅₅ solution at 214 nm: $t = 0$ h (A) and $t = 1$ h (B) after the initiation of conjugation reaction. Chromatographic separation was performed on a XBridge C18 (4.6 x 150 mm) column with 3.5 μ m particle size at 25 °C in a gradient separation from 5% to 100% AcN over 30 min. Solvents: H₂O (0.08 % TFA), AcN (0.08 % TFA).

Conclusions

The developed analytical methodology could be of great use in the chemistry and synthesis of glycopeptides. The optimum time needed for the termination of conjugation reaction was about six hours with yield more than 95%. In summary the conjugation reaction of a peptide (MOG₃₅₋₅₅) containing five extra lysines [(KG)₅MOG₃₅₋₅₅] with oxidised mannan polysaccharide was monitored *via* tricine gel electrophoresis. The termination of reaction was also confirmed *via* RP-HPLC. The presented methodology is a simple, fast and sensitive procedure that could be used in analytical chemistry for the detection of conjugates, peptides or proteins loaded into polysaccharides.

References

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