VAC.05 - Physical-chemical methodologies for molecular characterization of carrier proteins used in Bio-Manguinhos conjugated vaccines

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Introduction: Tetanus Toxoid (TT) and Diphtheria Toxoid (DT) are commonly used to confer T-dependent immune response in polysaccharide conjugate vaccines. In this sense, physical-chemical characterization is fundamental to assure product quality considering differences in production process, different suppliers or between batches of these proteins.

Objective: Evaluation of methodologies for molecular characterization of Tetanus Toxoid (TT) used in conjugate vaccines produced by Bio-Manguinhos.

Methodology: SEC chromatographic profile of two different samples of TT (TT1 and TT2) were performed using three different columns (Zorbax®GF450, TSK®G4000 SWXL and Superdex™ 200). Electrophoretic techniques such as SDS-PAGE, IEF-PAGE and NATIVE-PAGE were used to evaluate electrophoretic profile. Stability of secondary and tertiary structures were analyzed by circular dichroism and fluorescence spectroscopy respectively, with temperature varying between 25°C-85°C. All proteins were subjected to tryptic hydrolysis aiming obtain a peptide map by RPC chromatography. TT1 was used as standard for all analysis.

Results: SEC columns demonstrated to be effective for both samples, presenting two protein peaks (monomers and dimers). It was observed better resolution (RS>1.0) using Superdex™200, which was able to detect even a minor difference in molecular weight (4.75%) between TT1 and TT2. Besides, it was possible to observe better correlation (80.3%) between molecular masses obtained from the nominal value (150kDa). By SDS-PAGE, non-reduced TT1 and TT2 showed different profile with one band for TT1 referring the intact protein (126kDa) and three bands for TT2 related to intact protein, heavy and light chains (132, 100 e 46kDa respectively). After reduction, TT1 showed same profile of non-reduced TT2. These data suggest that TT2 presents three free polypeptides chains that are assembled on TT1. According to the literature, visualization of an intact protein in the presence of reducing agent could indicate that after the detoxification process some peptide chains had been covalently cross-linked by at least one non-reducible covalent bond. TT1 and TT2 presented a diffuse band by IEF-PAGE, with pI ranging from 4.75 to 5.0. Native-PAGE showed also a diffuse profile. Such data suggest the presence of isoforms with large variety in superficial charge and/or truncated forms probably generated during the detoxification process. Spectroscopic analysis showed that TT maintained its conformational stability until 60ºC. Secondary structure, with predominance of alpha helix was maintained over 85°C. The peptide map showed a different profile between hydrolyzed TT1 and TT2, corroborating with the data visualized on SDS-PAGE and SEC evidencing differences in the polypeptide chains of them.

Conclusion: Superdex™200, SDS-PAGE and peptide mapping were efficient to evaluate carrier proteins showing some molecular differences between analyzed samples. These methodologies were also useful to characterize the diphtheria toxoid, suggesting it could be effective for other carrier proteins. Moreover, other methodologies will be necessary to evaluate the impact of these molecular differences between carriers used in polysaccharide conjugation of the vaccines.

Keywords: conjugated vaccines; carrier proteins; Tetanus Toxoid