VAC.01 - Molecular characterization of tetanus toxoid used in conjugate vaccines produced by Bio-Manguinhos/FIOCRUZ

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Introduction:

Tetanus Toxoid (TT) is a widely used protein to confer T-dependent immune response in polysaccharide conjugate vaccines. In this sense, the TT molecular characterization is important to obtain a profile of this carrier in order to optimize the conjugation process and to ensure biosafety of the product once it is an injectable product.

Objective:

Structural and molecular characterization of TT used in Bio-Manguinhos conjugated vaccine using physico-chemical and biochemical methods.

Methodology:

TT was submitted to size exclusion chromatography-SEC (Zorbax GF450) and denaturing polyacrylamide gel electrophoresis (4-12%). Isoelectric point was estimated using polyacrylamide gel isoelectric focusing (IEF) with a pH range 3-9 and the stability of the secondary and tertiary structure were analyzed by spectropolarimetry of circular dicroism and fluorescence spectroscopy, varying temperature between 25°C-85°C.

Results:

TT presented two protein peaks by SEC with 262.7 ± 0.1 kDa (17.2±4.5 % area) and 143.5 ± 7.1 kDa (82.88 ± 4.5% area) suggesting dimeric and monomeric forms. By electrophoresis TT showed two bands with 141.62 and 115.17 kDa in the absence of DTT, and three bands with 147.42, 111.91 and 50.96 kDa using DTT. By IEF, TT presented a diffuse band with pI ranging from 4.75 to 5.0. Such data could be explained by the presence of truncated TT forms generated during detoxification process. The spectroscopic analysis showed that TT maintained its conformational stability until 60°C and its secondary structure over 85°C, as expected to a vaccine carrier.

Conclusion:

These data showed that TT is a stable protein. The presence of truncated forms of TT makes it mandatory to use different techniques to characterize the carrier to be used in polysaccharide conjugation processes. The methodologies applied were useful in the characterization of TT used in this study.

Keywords: conjugated vaccines; carrier proteins; tetanus toxoid