6-12-2022

# New insights in formaldehyde-induced detoxification of the tetanus toxin: Chemical modification stoichiometry and characterization of intra- and inter-molecular cross-links

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# NEW INSIGHTS IN FORMALDEHYDE-INDUCED DETOXIFICATION OF THE TETANUS TOXIN: CHEMICAL MODIFICATION STOICHIOMETRY AND CHARACTERIZATION OF INTRA- AND INTER-MOLECULAR CROSS-LINKS

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Key Words: tetanus toxoid , mass spectrometry, formaldehyde modifications, cross-link.

The active ingredient of the tetanus vaccine, known as tetanus toxoid (TTD, 150 kDa), is prepared by inactivation of the tetanus toxin with formaldehyde. This chemical treatment determines the efficiency and innocuity of the vaccine. The detoxification chemistry has been qualitatively studied regarding the nature and positioning of some formaldehyde-induced modifications. However, neither the stoichiometry of the chemical modifications or intra- and inter-chain cross-linking have been yet characterized, despite being of great interest for the definitive elucidation of the detoxification mechanism as well as for future manufacturing follow-up. The aim of the present study is to assess the impact of formaldehyde on TTD by identifying, mapping, and quantifying as exhaustively as possible, all formaldehyde-induced modifications over the entire three-dimensional (3D) structure of the protein.

The analytical strategy was very effective and allowed to identify ≥100 formaldehyde modifications. These modifications were localized on 70 amino acid residues of lysines, tyrosines, asparagines, tryptophanes, glutamines, arginines and histidines. Several methylene-type cross-links were unambiguously characterized for the first time in the subunits and across the light and heavy TTD. After identifying the nature and location of all formaldehyde-induced modifications on the primary sequence of TTD, we mapped them over the entire 3D structure of the protein.

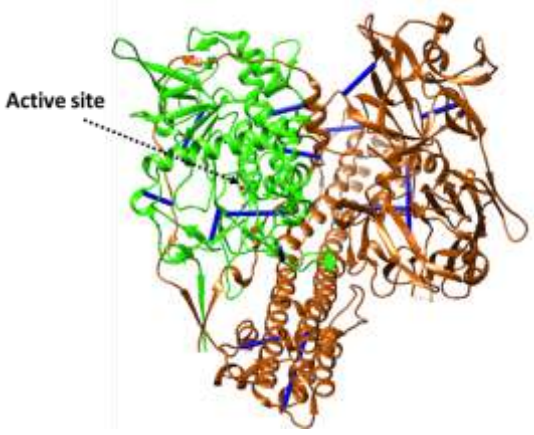


Figure : crystalline structure of tetanus toxin. Formaldehyde-induced cross links are colored in blue.

A label-free quantification approach based on the reconstructed peak area from the first MS dimension of both the non- and chemically-modified forms of each peptide was performed to estimate the rate of formaldehyde-induced modifications in TTD.

Detoxification of the toxin at the molecular level could be explained by some (if not all) formaldehyde-induced modifications characterized in this study. The inter-chain cross-links could explain why the light and heavy chains linked with a disulfide bridge do not dissociate after reducing treatment, thus supporting TTD safety.