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Polysorbate 20 and 80 Degradation by Group XV Lysosomal Phospholipase A₂ Isomer X1 in Monoclonal Antibody Formulations

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ABSTRACT

Decreases in the intact polysorbate (PS-20 and PS-80) content were observed while evaluating the long-term storage stability of CHO derived, purified monoclonal antibodies. It was determined that polysorbate had been enzymatically degraded; therefore, studies were performed to identify and characterize the protein(s) responsible. Polysorbate degrading activity was enriched from CHO media leading to the identification of Group XV phospholipase A₂ Isomer X1 (PLA2) by LC-MS/MS. Recombinant phospholipase A₂ was expressed, purified and conformational integrity confirmed against a phosphatidylcholine substrate. Incubation of recombinantly produced PLA2 with PS-20 and PS-80 resulted in hydrolysis of both monoester and higher order PS-20 and PS-80 but a much slower rate was observed for higher order PS-80. Endogenous phospholipase A₂ was detected and quantitated at less than 1 ppm in three formulated antibodies while phospholipase A₂ was not detected (or less than 0.1 ppm) in a fourth formulated antibody. Furthermore, antibodies with detectable quantities of endogenous phospholipase A₂ demonstrated polysorbate hydrolysis while in contrast the antibody without detectable phospholipase A₂ did not show polysorbate hydrolysis. Comparison of polysorbate degradation products generated from the formulated antibody and samples of polysorbate incubated with recombinant phospholipase A₂ resulted in similar elution profiles by LC-MS. These results suggest that phospholipase A₂ may play a key role in polysorbate degradation in some antibody preparations.

Key words: Polysorbate 20, polysorbate 80, lipase, Group XV Lysosomal Phospholipase A₂ Isomer X1, antibody, hydrolysis