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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 A_2 Isomer X1 (PLA2) by LC-MS/MS. Recombinant phospholipase A_2 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 A_2 was detected and quantitated at less than 1 ppm in three formulated antibodies while phospholipase A_2 was not detected (or less than 0.1 ppm) in a fourth formulated antibody. Furthermore, antibodies with detectable quantities of endogenous phospholipase A_2 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 A_2 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 A_2 Isomer X1, antibody, hydrolysis