Lysozyme inactivation by free radicals can occur independently of reactive carbonyl formation

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The relationship between the free radical-induced loss of lysozyme activity and the occurrence of oxidative modifications, evidenced as gain in DNPHreactive carbonyl groups (CO) and loss of tryptophan-associated fluorescence (TAF), was addressed in vitro.2,2'-azobis (2-amidino propane)(20 raM, AAPtt) and a mixture of Fe+2/Ascorbate (Fe+2/A, 0.1/25 mM) were used as peroxyl and oxygen-de rived free radical sources. Lysozyme incubated (2h, 37C) in the presence of AAPH underwent a 75% decrease in its activity, a concomitant 50% loss of TAF, and a 6-fold rise in CO levels. Such a change in CO levels, however, could account at the most for the inactivation of 6% of the enzyme molecules. Further suggesting a dissociation between CO formation and inactivation, the enzyme's activity and the TAF remained totally unaltered upon exposure of the protein to Fe+2/A in spite of an almost 5-fold rise in CO. The effects induced by either AAPH or Fe+2/A were all inhibitable by antioxi dant addition. Neve