

Stability of Proteins encapsulated in Poly(lactic-co-glycolic acid) Delivery Systems

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The instability of protein therapeutics and vaccine antigens encapsulated in the biodegradable controlled-release polymer-of-choice, poly(lactic-co-glycolic acid) (PLGA), is the single most important issue in the development of these delivery systems. Numerous deleterious stresses causing protein damage during microencapsulation, storage, and release from PLGA have been identified. Among them, the microenvironment within PLGA (i.e., elevated moisture and temperature, and variable pH) during protein release is considered most significant. Model proteins were employed to: i) identify the most significant stresses during protein release, ii) elucidate molecular mechanisms of instability, and iii) develop general approaches to stabilize encapsulated proteins. For example, using bovine serum albumin (BSA), it was demonstrated that PLGAs frequently develop an acidic microclimate, which induces BSA non-covalent aggregation and hydrolysis. BSA was successfully stabilized by neutralizing the low pH with co-encapsulated antacid excipients (e.g., MgCO_3). Another important example is the employment of BSA, which was formaldehyde-treated to yield formalinized BSA (f-BSA) similarly as bacterial toxins are routinely detoxified to yield the corresponding toxoids. The model formalinized antigen exhibited similar aggregation behavior in the solid state as observed with bacterial vaccine antigens, tetanus and diphtheria toxoids. By using fBSA, and the unformalinized BSA as control, a unique formaldehyde-mediated aggregation pathway (FMAP) was proven and characterized. The free amino acids, histidine and lysine, which strongly interact with formaldehyde, were identified to prevent the FMAP for PLGA-encapsulated fBSA when the amino acids were co-encapsulated with the antigen. The two mechanistic approaches were found to stabilize numerous PLGA-encapsulated therapeutic proteins and vaccine antigens including basic fibroblast growth factor (bFGF) and tetanus toxoid. Stabilized PLGA formulations of bFGF were found to be angiogenic in vivo.

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