505g Dft Study and Kinetic Model of Deamidation: Applications to Protein Stabilization

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Protein deamidation is an important obstacle in the development of therapeutic proteins, particularly for increasingly common treatments using monoclonal antibodies. A mechanistic understanding would be useful in designing protein formulations that are stable to degradation by deamidation. We present a detailed DFT study of a network of elementary reactions to explain the mechanism of asparagine deamidation. At low pH, deamidation occurs by direct acid catalyzed hydrolysis of asparagine. At neutral to basic pH, deamidation proceeds by the initial formation of an anionic intermediate. The anionic intermediate can be converted to succinimide by three separate pathways that shift in relative importance with pH. The calculated rate constants from each pathway were incorporated into a kinetic model. The overall rate constant was then computed as a function of pH to obtain an apparent first order rate constant for asparagine deamidation. The calculated rates show four distinct regimes of pH dependence like the regimes experimentally observed by Capasso et al. These results support our hypothesized mechanism of elementary steps.