

The Essential Need for Protein Chemists

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Although still a comparatively small overall percentage, protein therapeutics (protein “biopharmaceuticals”) are the fastest-growing category of new drugs approved by the U.S. FDA (1,2). In 2002 the U.S. Biotechnology Industry Organization reported FDA approval of 130 biotechnology-derived proteins, with over 350 in additional clinical trials. By 2005 the number of FDA approved protein biopharmaceuticals had almost doubled, and currently target over 200 human diseases, including cancers, heart disease, Alzheimer’s, diabetes, Multiple Sclerosis, AIDS, and arthritis (2). Recent data shows that there are over 1,500 biotechnology companies in the U.S. alone, with a market capitalization in excess of \$410 billion (2). The impact of protein biopharmaceuticals upon global healthcare and economy is substantial and growing rapidly.

Proteins are a novel type of compound in comparison to traditional small molecule pharmaceuticals, and present new and significant challenges to the realization of their full potential as therapeutics. One fundamental difference is that proteins are potentially capable of adopting different structural conformations, and this can profoundly influence critically-important properties such as function, solubility, bioavailability, half-life, aggregation, toxicity, and immunogenicity (3-5). In the simplest case, proteins can exist in either a native (folded) or denatured (unfolded) state. The native state is associated with function and is generally considered to represent the global Gibbs energy minimum. The denatured state (an ensemble of “disordered” structures) is associated with loss of function and (under normal physiological conditions) is higher in Gibbs energy. The transition between these states is a physical process (modulated by heat, pH or solutes), and thus potentially reversible, with the partition function defined by the Gibbs energy difference ($\Delta G_{\text{unfolding}}$). The formal “thermodynamic stability” (or thermostability) of a protein refers to the magnitude of $\Delta G_{\text{unfolding}}$, an intrinsic property of the protein. This simplified view becomes more complex when the properties of the denatured (or

partially-denatured) state are considered. The denatured state typically has reduced solubility (and potential residual structure) that can promote aggregation, resulting in an irreversible pathway from the denatured state. The denatured state is also much more accessible to proteolytic attack and chemical modification, which contribute to additional irreversible processes leading from the unfolded state. Thus, while reversible folding is associated with recovery of function, irreversible processes are often the primary determinants of observed functional half-life (often referred to in the literature as “functional stability”). From this simplified analysis it can be appreciated that the formal thermodynamic stability and the observed functional stability of a protein are distinct but inter-related.

The details of thermodynamic and functional stability are of prime relevance when aggregated forms of the protein are toxic, or result in the generation of novel immunogenic epitopes. Neutralizing antibodies in patients treated with interferon-alpha 2a were observed when the protein was stored at room temperature and formed detectable aggregates. Consequently, both the formation of aggregates and immunogenicity were reduced upon storage at 4 °C (6). Persistent antibodies were generated in patients treated with natural human growth hormone with formulations containing 50-70% aggregates; however, when the formulation was modified to result in <5% aggregates, only transient or no antibodies were observed (7). In another study of recombinant clotting factor VIII in mice, the formation of aggregates was associated with the emergence of entirely novel immunogenic epitopes (8). Neutralizing antibodies can prove fatal when they cross-react with the endogenous form of an essential protein. Thus, protein denaturation, aggregation and immunogenicity are critical interrelated issues for protein biopharmaceuticals.

All of these data point to an essential need for well-trained protein chemists to realize the vast potential of biotherapeutics. To be most effective, such training should be broad, and include physical chemistry,

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thermodynamics, enzymology, folding kinetics, structural biology, medical biochemistry and augmented with molecular modeling and computational science including bioinformatics. While these training demands are both extensive and demanding, they permit the thorough analyses essential for solving the challenges in the effective application of protein biotherapeutics. Given the vast potential impact of protein therapeutics on human health and well-being, the rewards of success are correspondingly great. The training of protein chemists is critical.

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