Protein Folding, Misfolding, Stability, and Aggregation

An Overview

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Protein misfolding and aggregation problems arise in diverse arenas. In the manufacture of commercial protein products, correctly folded proteins in stable formulations are critical for safety and efficacy. In the clinic, there is increasing awareness that protein aggregation is an underlying cause of several severe and chronic diseases. This chapter provides a brief background on the nature of folded and misfolded proteins and on the forces that drive protein folding, misfolding, and aggregation. We then give an overview describing the organization and contents of the remainder of this volume.

1. IMPORTANCE OF PROTEIN MISFOLDING AND AGGREGATION

1.1. Manufacture of Protein Products

In a typical biopharmaceutical production process there are many points at which protein aggregation can occur. A protein expressed in Escherichia coli often aggregates shortly after it is synthesized. With varying degrees of success, correctly folded active protein can be recovered by solubilization and refolding. When a recombinant protein is made in mammalian cells, the cellular machinery can process the protein such that solubility is often maintained at the time of harvest. However, successful synthesis in the bioreactor is far from a guarantee of a soluble final drug substance. Unwanted aggregation is a common by-product of rigorous purification processes. For instance, monoclonal

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antibodies are often purified using protein A chromatography that requires acid pH for elution. Viral inactivation required for mammalian cell-derived products is carried out in even harsher acidic conditions. Acid denaturation of the protein product as well as other host cell biomolecules leads to aggregation and loss of product. Depth filtration, diafiltration, and other similar processes can cause aggregation due to shear-induced protein denaturation. A protein product that survives purification processes then must be formulated for a useful shelf life, to maintain a drug’s stability until the point of administration. Cosolutes and other excipients often are recruited to improve a product’s stability. As a greater diversity of proteins reach the market, the industry must incorporate new understanding of mechanisms of protein misfolding and aggregation in order to develop robust manufacturing and formulation processes that result in stable, correctly folded, and active products.

1.2. Protein Misfolding Diseases

The problem of protein structural instability, misfolding, and aggregation is not limited to manufacturing. The “protein misfolding” diseases constitute a newly recognized group of diseases with a diverse array of symptoms. Some of the most common protein misfolding diseases are neurodegenerative, including Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, and the prion diseases. These diseases share a common feature: the deposition of insoluble, usually fibrillar, β-sheet-rich protein aggregates. The source and nature of the aggregating protein, the location of the deposit, and the biological consequences differ from disease to disease (Table 1). The factors that trigger formation of aggregates and the mechanisms by which aggregation leads to disease are poorly understood. Development of effective treatment and prevention therapies for these diseases requires elucidation of the molecular basis for protein misfolding and aggregation.

2. PROTEIN STRUCTURE AND PROTEIN FOLDING

2.1. Amino Acids

Twenty amino acids make up the library from which natural proteins are synthesized (Table 2). Within their side chains is contained a wide diversity of chemical function. Table 3 summarizes important physicochemical properties of these amino acids. At a fundamental level, these properties direct the folding, misfolding, and aggregation of proteins. It should be noted that many different scales have been proposed to measure the relative hydrophobicity of the side chains.

Covalent modifications of these side chains by reactions such as phosphorylation, glycosylation, oxidation, or deamidation introduced by design or by accident further increase chemical diversity and affect protein structure and stability. In a manufacturing environment, the ability to control, minimize, or completely eliminate these modifications directly contributes to the quality attributes of a product. For instance, the glycosylation pattern of a monoclonal antibody can be part of the release specifications and one of