Physical Stabilization of Proteins in Aqueous Solution

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INTRODUCTION

The formulation scientist’s key goal is to achieve long-term stability of a drug compound. In the case of protein drugs, stabilization means not only maintaining the native chemical structure, but the native secondary and higher order structures necessary for biological activity (Cleland et al., 1993; Manning et al., 1989). Denaturation, as it is defined in this context, will be the process of forming any non-native physical or chemical state of the protein. Physical and chemical denaturations are often accompanied by covalent and non-covalent aggregates that not only can destroy the activity of the drug, but also cause adverse side effects (Carpenter and Chang, 1996; Thornton and Ballow, 1993). Without the ability to stabilize native protein structures, even the most efficacious protein therapeutics will fail to make viable drug products.

How does a formulation scientist develop a formulation that stabilizes native protein structure against physical and chemical stresses in solution, and what are the relevant stresses that cause denaturation? These are the questions that this chapter will address.
Although the chemical and physical stabilities of a protein may seem separate parameters, they are actually closely tied to one another (Brange, 1992; Khosravi et al., 2000; McCrossin et al., 1998; Rahuel-Clermont et al., 1997). Physical degradation of a protein can lead to covalent changes (oxidation, hydrolysis, disulfide scrambling). The reverse is also true; reduction of disulfide bonds, hydrolysis, and other covalent changes can cause a loss of the protein native state. For chemical degradations that are linked to physical degradation, formulations that stabilize the native state will necessarily stabilize against the chemical degradation. Understanding these relationships between physical and chemical stabilities is currently a major goal in formulation research.

OVERVIEW OF PHYSICAL STABILITY

The physical stability of a protein relates to maintaining the native secondary and higher order structures. To understand how to use formulation variables to stabilize the native structure, one must understand the mechanisms by which a protein adopts a folded conformation in solution. These mechanisms may be separated into thermodynamic and kinetic contributions to protein stability (Baker and Agard, 1994; Jaenicke, 1995).

Thermodynamic Control of Protein Stability

The traditional techniques of equilibrium unfolding have led to a vast amount of information on the thermodynamics involved in protein stability (Pace, 1975; Privalov, 1979; Schellman, 1987; Tanford, 1968). Equilibrium unfolding is typically accomplished by varying temperature or pH, or by adding denaturants such as guanidine hydrochloride or urea (Pace, 1975). To compare the stabilities of various proteins, the energetics of the unfolding reactions are typically extrapolated to conditions where the proteins exist predominantly in the native state (neutral pH, room temperature, isotonic, etc.) These techniques have given information on the enthalpic and entropic forces that stabilize the solution native state of proteins, and have generally shown that the free energy of stabilization of the native state is typically 5–20 kcal/mol (Dill, 1990; Privalov, 1979). Further information on the forces involved in protein stability has been obtained by unfolding under varying solution conditions (i.e. different pH, salt concentrations, hydrogen-bond competing molecules, etc.). The results of these experiments, coupled with experiments on model peptides, has led to an understanding of the contribution of ionic, hydrogen bonding and hydrophobic effects role in protein stability (for a review, see (Dill, 1990)).

Hydrophobic interactions are most likely the dominant forces in stabilizing the native structures of proteins (Dill, 1990; Kauzmann, 1959; Schellman, 1987).