Rational Design of Stable Protein Formulations

Theory and Practice

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Surfactant-Protein Interactions

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INTRODUCTION

To retain biological activity, proteins generally must be maintained in a specific, three-dimensional conformation. This conformation is only marginally stable, and thus relatively minor perturbing forces can disrupt protein structure, causing loss of biological activity, as well as formation of non-native protein aggregates. Such perturbations are commonly encountered as proteins are produced, stored, transported, and delivered to patients. For example, it is well known that during common industrial processes such as filtering (Maa and Hsu, 1998), storage (Mcleod et al., 2000), agitation (Thurow and Geisen, 1984; Maa and Hsu, 1997) freeze/thawing (Eckhardt, Oeswein et al., 1991; Nema and Avis, 1993; Izutsu et al., 1994), lyophilization (Carpenter and Chang, 1996; Carpenter et al., 1997), nebulization (Ip et al., 1995) and spray-drying (Broadhead et al., 1994; Mumenthaler et al., 1994; Maa et al., 1998; Adler and Lee, 1999; Millqvist-Fureby, Malmsten et al., 1999; Tzannis and Prestrelski, 1999) proteins can suffer damage to their native conformation. Further, delivery of protein pharmaceuticals to patients may also provoke losses of conformational integrity via unfavorable interactions of proteins with surfaces (e.g., inner walls of catheter tubing or syringes (Tzannis et al., 1996)).

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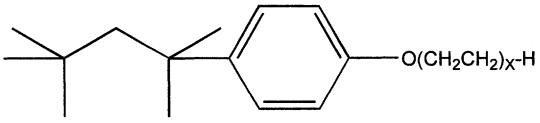
The mechanisms of degradation of protein structure and activity are often categorized in two broad classes, chemical and physical. Chemical degradation refers to those modifications involving covalent bonds, such as deamidation, oxidation and disulfide bond shuffling. Physical degradation includes unfolding of the protein, undesired adsorption of the protein to surfaces, and aggregation. The two categories are not completely independent of one another. For example, protein oxidation may result in a greater proclivity to aggregate, and the rate of non-native disulfide bond formation may be higher in aggregated proteins.

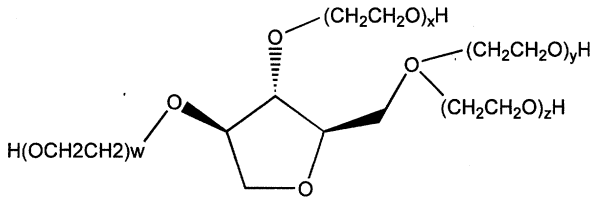
Surface-active agents, or surfactants, are often added to protein solutions to prevent physical damage during purification, filtration, transportation, freeze-drying, spray-drying and storage. Surfactants are amphiphilic, containing a polar head group and a non-polar tail. This dual nature causes surfactants to adapt specific orientations at interfaces and in aqueous solutions. It is this characteristic that lies at the root of the mechanisms by which surfactants affect the physical stability of proteins.

A well-known example is the anionic surfactant sodium dodecyl sulfate, or SDS. The sulfate anion is the hydrophilic head group of SDS, while the long aliphatic dodecyl chain forms the tail group. Ionic surfactants such as SDS have been known since the late 1930's as effective protein denaturants (Anson, 1939), and are commonly used for this purpose, e.g., as a pre-treatment for proteins in polyacrylamide gel electrophoresis (SDS-PAGE). In contrast, surfactants used as stabilizing agents in protein formulations are typically non-ionic (Loughheed et al., 1983; Twardowski et al., 1983; Chawla et al., 1985). This chapter will focus on non-ionic surfactants; protein interactions with ionic surfactants have been reviewed elsewhere (Jones, 1996). An example non-ionic surfactant is polyoxyethylene sorbitan monolaurate (Tween 20®), shown in Figure 1. In this molecule, the hydrophilic polyoxyethylene units form the head group, while the hydrophobic monolaurate group is the tail. Tween 20 is often added to formulations due to its ability to protect proteins from surface-induced denaturation (Chang et al., 1996; Jones et al., 1997; Bam et al., 1998; Kreilgaard et al., 1998; Maa et al., 1998).

There are a number of mechanisms by which surfactants can prevent or promote damage to proteins. Some of these mechanisms are generic to all excipients, and can be explained in the solution thermodynamic framework of the Wyman linkage theory (Wyman and Gill, 1990) and the preferential exclusion mechanisms developed by Timasheff and colleagues (Arakawa and Timasheff, 1982, 1983, 1984a,b, 1985a,b,c; Arakawa et al., 1990; Timasheff, 1998). Others derive from the amphiphilicity of surfactants and the resulting effect of microscopic ordering of surfactant molecules at interfaces, which in turn affects the kinetics and thermodynamics of protein interfaces. In this chapter, we discuss a number of these mechanisms and their implications for protein stability.



Polyethylene glycol ether
Triton X-100, x=9-10 (average)
Triton X-114, x=7-8 (average)



w+x+y+z=20
Polysorbate
Tween 20, R=$C_{11}H_{23}CO_2$
Tween 80, R=$C_{17}H_{33}CO_2$

Figure 1. Example non-ionic surfactants.

PROTEINS AND SURFACTANTS AT SURFACES

Because of their dual hydrophobic/hydrophilic nature, surfactants in solution tend to orient themselves so that the exposure of the hydrophobic portion of the surfactant to the aqueous solution is minimized. Thus, in systems containing air/water interfaces, surfactants will tend to accumulate at these interfaces, forming a surface layer of surfactant oriented in such a fashion that only their hydrophilic ends are exposed to water. Such orientation and surface adsorption can also occur at solid/water interfaces such as those found in vials, syringes, and other containers. Protein molecules also exhibit surface activity (for a review see (Magdassi, 1996), and references therein) and as such will also tend to adsorb to and orient at these interfaces.

From classical thermodynamics, the excess surface internal energy dU_1^{σ} of a surface with area A at a temperature T is related to the excess surface entropy

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