7

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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Apotex Exhibit 1027
Page 1 of 17

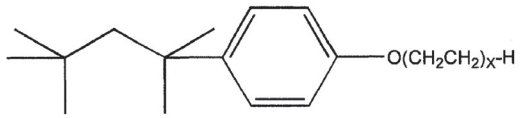
The mechanisms of degradation of protein structure and activity are often categorized in two broad classes, chemical and physical. Chemical degradation refers to those modifications involving covalent bonds, such as deamidation, oxidation and disulfide bond shuffling. Physical degradation includes unfolding 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.

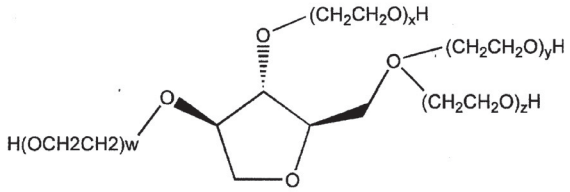
A well-known example is the anionic surfactant sodium dodecyl sulfate, or SDS. The sulfate anion is the hydrophilic head group of SDS, while the long aliphatic dodecyl chain forms the tail group. Ionic surfactants such as SDS have been known since the late 1930's as effective protein denaturants (Anson, 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.

Apotex Exhibit 1027
Page 2 of 17



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

Apotex Exhibit 1027
Page 3 of 17

S_1^σ and the chemical potential and number of surface excess moles of each adsorbed species:

$$dU_1^\sigma = TdS_1^\sigma + \sigma dA + \sum_{i=2}^{c} \mu_i dn_{i,1}^\sigma \tag{1}$$

Here the subscript 1 refers to a dividing surface chosen so that there is no excess adsorption of species 1, the solvent (water) (Gibbs, 1961), and σ is the surface tension. The equilibrium criterion, $\delta S = 0$, requires that σ be constant across the surface. Thus, if a protein adsorbs to an interface, at equilibrium the surface tension forces must be continuous and constant across the whole interface, including across the protein. The stability criterion at equilibrium requires that:

$$\left(\frac{\partial \sigma}{\partial A}\right)_{T,n} > 0 \tag{2}$$

If the surface tension of the interface is greater than the internal tension in the protein, then in order to meet these two conditions, the surface area of the protein must increase until the two tensions are equal, i.e., the protein must unfold. In some cases, nearly complete loss of native activity is lost upon adsorbing to the interface (Rothen, 1947; Verger et al., 1973). The Gibbs adsorption equation relates the surface tension to the concentration of adsorbed species at an interface:

$$-d\sigma = s_1^\sigma dT + \sum_{i=2}^{c} \Gamma_{i,1} d\mu_i \tag{3}$$

where $\Gamma_{i,1}$ and $s_1^\sigma dT$ are, respectively, the excess surface adsorption and excess surface entropy of component *i*, both relative to a dividing surface with no surface excess of solvent (1), and μ_i is the chemical potential of species *i*. Adsorption of protein to the interface thus lowers the interfacial tension, making unfolding less likely as adsorption progresses.

If the process of surface adsorption and unfolding of protein were to stop after the formation of an equilibrium monolayer, the amount of adsorbed protein would be so small as to be generally of no consequence. Indeed, a significant amount of work has been dedicated to development of methods with sensitivities high enough to characterize the minute amount of protein adsorbed to the interface (Tupy et al., 1998; Vermeer and Norde, 2000). However, depending on the degree of surface hydrophobicity and characteristics of the protein in question, additional processes can occur in the adsorbed films, leading to behavior that is

Apotex Exhibit 1027
Page 4 of 17

no longer described by the Gibbs' adsorption equation. Among the possible processes are gas-to-liquid surface phase transitions, surface precipitation, and the formation of surface sublayers. In many cases, the adsorbed molecules can be rapidly released from the surface and surface sublayers, and may exchange with bulk protein molecules. Alternatively, because of slow refolding kinetics, proteins can become irreversibly adsorbed at the interface, or have a rate of exchange somewhere between these extremes (Dickinson, 1999; Norde and Giacomelli, 1999). These processes of adsorption and release of structurally-perturbed protein molecules into the bulk solution have been implicated as one of the causes of protein aggregation and denaturation.

When discussing protein adsorption at interfaces, globular proteins are typically characterized as being either "hard" or "soft," having a low or high degree of flexibility, respectively, and by their degrees of hydrophobicity. Soft, hydrophobic proteins, attaining monolayer coverage of the air/water interface in the matter of minutes, are generally more surface reactive at hydrophobic surfaces than hard, hydrophilic proteins, attaining coverage of the same surfaces in a matter of hours (Tripp et al., 1995). The driving force for protein adsorption is the decrease in the entropy of the water molecules that are ordered around the hydrophobic protein domains when the protein is in the bulk solution. Thus, the role of the relative degree of hydrophobicity on protein surface adsorption is rather straightforward: given two proteins only differing in their hydrophobicities, the more hydrophobic protein will have a greater number of productive interactions with the surface and will form the monolayer more quickly. Middelberg et al. (2000) proposed that the difference in adsorption kinetics of Lac21 and Lac28 peptides is because the monomeric Lac21 has more hydrophobic residues exposed than its tetratmeric counterpart, Lac28; thus, Lac21 more readily forms a monolayer at an octane-water interface. Protein flexibility is important in protein spreading that occurs at the interface. A flexible protein can expose additional non-polar residues, leading to an increased strength in binding to the surface. Finally, the protein flexibility dictates the number of proteins that can adsorb at the interface, and their spreading rate (Norde and Giacomelli, 1999).

Protein adsorption to a hydrophobic surface does not necessarily lead to a *complete* loss of "native" structure: some proteins actually gain structure. For example, melittin, a honeybee venom peptide, increases its α-helical content slightly when adsorbed to hydrophobic quartz. In contrast, adsorption of a tetramer of the same peptide is thought to require a loosening of the α-helical content. The orientation of the adsorbed helices in the peptide is parallel to the quartz plane, with the hydrophobic moieties facing the plane (Smith and Clark, 1992). Caessens et al. (1999) also report an increase in helical content on the adsorption of the predominately random coil β-casein and β-casein peptides at the teflon/water interface.

Apotex Exhibit 1027
Page 5 of 17

Surfactants will also adsorb to the interface. The Gibbs adsorption isotherm again predicts that this interaction will lower the surface tension. Thus, if surfactants co-adsorb to an interface together with proteins, there will be a smaller driving force for surface adsorption. Furthermore, competition between the protein and the surfactant for the interface may reduce equilibrium protein adsorption. For example, Tween 20 addition displaces beta-lactoglobulin films from air-water interfaces (Roth et al., 2000). This effect is not universal: the ionic surfactant sodium dodecyl sulfate forms a complex with high surface activity that exhibits enhanced surface adsorption (Green et al., 2000). Finally, because the surface tension is lowered after surfactant is adsorbed, less damage to the protein that does adsorbed may occur. For example, a loss of α-helix and an increase in β-turn structures occur when bovine serum albumin adsorbs to polystyrene particles. This effect is decreased when the surface is more crowded (Norde and Giacomelli, 2000).

Protein stabilization by nonionic surfactants can often be observed by formulating with micromolar concentrations of surfactant. This is due to the high surface-activity of this class of excipients, which renders a higher effective concentration of surfactant molecules at interfaces than in the bulk solution. When the concentration of the surfactant is much lower than its critical micelle concentration (CMC), surfactant molecules lie flat at the air/water and hydrophobic solid/water interfaces (Figure 2A) (Porter, 1994). As the concentration is increased, more molecules adsorb to these interfaces, such that the surface concentration remains linearly proportional to the bulk concentration (Tanford, 1973). This crowding forces the surfactant molecules to order themselves such that the hydrophilic groups are oriented towards the bulk water and the hydrocarbon chains are pointed towards the air or hydrophobic solid (Porter, 1994; Fainerman et al., 2000) (Figure 2B). At sufficiently high surfactant concentrations (i.e., at or above the CMC), there is an oriented monolayer of surfactant molecules and maximum surfactant absorption, at the interface (Figures 2C & D). The surface saturation is responsible for the sharp slope change (to essentially zero) observed in experimental plots of surface properties (surface tension, osmotic pressure) versus surfactant concentration (Porter, 1994) (Figure 3). It should be noted that the linear variations in the surface properties shown in Figure 3 are indicative that the surface activity of a surfactant is due to the hydrophobic effect: the variation would be cooperative if hydrocarbon self-affinity was the appropriate explanation (Tanford, 1973). Surfactant micelles are formed in the bulk phase when the concentration is of the surfactant is above the CMC (Figure 2D). Thus, a range of surfactant concentrations could inhibit protein denaturation at an interface; however, the necessity of CMC levels of surfactant to completely inhibit protein damage would be a strong indication that the damage is caused by adsorption of protein at the interface and that inhibiting protein adsorption at the interface plays a role in preventing protein aggregation.

Apotex Exhibit 1027
Page 6 of 17

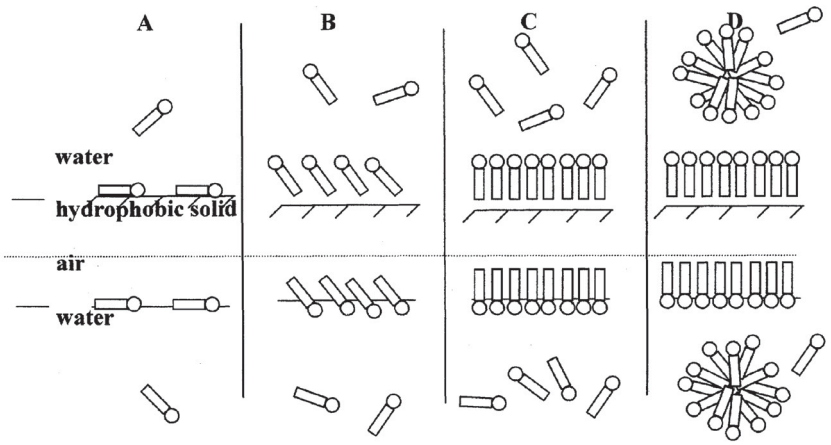


Figure 2. Simplified models of the interfacial behavior of a nonionic surfactant at several concentrations in water. Circles—polar head groups (e.g., polyoxyethelenes). Rectangles—hydrophobic tails (e.g., hydrocarbon chains). Models above the dotted line is for the hydrophobic solid (ice)/water interface and those above are for the air/water interface. (**A**) Surfactant concentration is well below the CMC (**B**) Surfactant concentration is greater than in **A**, but still below the CMC. (**C**) Surfactant concentration is at the CMC. (**D**) The surfactant concentration is above the CMC. (This model is adapted from Figures 4.4 and 4.8 of Porter (Porter, 1994)).

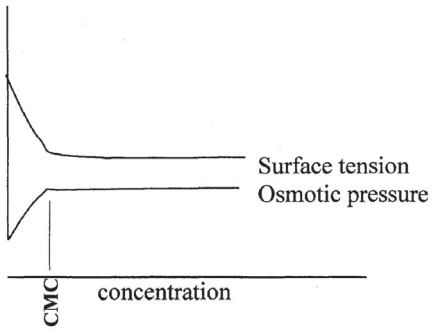


Figure 3. Representation of changes two properties in determining the CMC of a surfactant.

Decreased protein adsorption at interfaces (e.g., air/liquid, ice/liquid) in the presence of nonionic surfactants such as Tween 20 can be attributed to the surface activity of nonionic surfactants (Chang et al., 1996; Kreilgaard et al., 1998; Miller et al., 2000a,b) and, in some cases, direct interactions between the surfactant and protein molecules (Dickinson, 1998; Bam et al., 1998; Miller et al., 2000a,b). In

Apotex Exhibit 1027
Page 7 of 17

mixed protein/surfactant systems in which the surfactant binds to hydrophobic regions of the protein, the protein is less surface-active than it would be in a solution devoid of the nonionic surfactant. This explains the increase in surface tension relative to that of the pure protein solution that can be observed at extremely low surfactant concentrations. Adsorption of surfactant and protein molecules in the mixed system is competitive. Nonionic surfactants usually bind tighter than proteins or protein–surfactant complexes at interfaces (Dickinson, 1998). Thus, above a critical concentration of the surfactant, protein adsorption becomes negligible and the adsorption isotherms for mixed surfactant/protein systems can be roughly identical that of a pure surfactant solution, as observed by Miller et al. (2000a) for the HSA/C_{10}DMPO when the C_{10}DMPO concentration exceeds 10^{-7} mol/cm^3.

PROTEIN-SURFACTANT INTERACTIONS IN SOLUTION

In addition to altering the interaction of proteins with surfaces, non-ionic surfactants can also interact directly with proteins in solution. For example, Tween 20 acts as a chemical chaperone, aiding in the refolding of proteins (Bam et al., 1998; Kreilgaard et al., 1999). *In vivo*, proteins fold while at average concentrations of approximately 35 mg/ml (Hartl, 1996): *in vitro*, non-native protein molecules at this concentration (e.g., due to freeze concentration) usually aggregate. Inside cells, protein folding is aided by naturally occurring molecular chaperones. Unlike folding catalysts that have steric information to guide the protein folding, molecular chaperones act by non-covalently binding to partially-folded proteins, usually via hydrophobic interactions, to prevent misfolding or aggregation while the protein is attempting to adopt its native conformation (Gatenby and Ellis, 1990; Hartl, 1996). Chaperone-assisted protein refolding can prevent the protein from falling into kinetic traps or simply allow more time for the protein to refold (Gatenby and Ellis, 1990). The hydrophobic effect, the driving force of protein folding and surface activity of Tween 20, is also implicated in the interactions between the exposed hydrophobic regions of the partially folded protein and the hydrocarbon tail of the surfactant. These mixed surfactant/protein complexes and protein folding are dynamic processes, eventually the protein attains a conformation in which the hydrophobic groups are not as surface exposed. Unless there are hydrophobic patches in the native protein conformation, the surfactant molecules will not necessarily bind to the protein, as in the case of molecular chaperones. *In vitro*, surfactants such as Tweens (Bam et al., 1996), polyethylene glycol (Cleland and Wang, 1990; Cleland et al., 1992; Cleland and Randolph, 1992; Cleland 1993; Cleland and Wang, 1993), Triton

Apotex Exhibit 1027
Page 8 of 17

X-100 (Donate et al., 1998), and lubrol (Donate et al., 1998), have been implicated in aiding protein refolding by acting as chemical chaperones.

Surfactants, such as Tween 20, can also affect the thermodynamic conformational stability of a protein. As discussed in other chapters in this book, and in detail in references from Timasheff and co-workers (Lee and Timasheff, 1981; Arakawa and Timasheff, 1982; Arakawa and Timasheff, 1985; Timasheff, 1998), thermodynamic stability is increased if a ligand exhibits greater binding to the native state of a protein than to a non-native state. However, with many excipients (e.g., sucrose) excluded volume effects produce a non-specific negative binding to the native state, and a concomitantly larger negative binding to expanded, non-native conformations. This differential negative binding also results in a stabilization of the native state. At the low concentrations of surfactant (*ca.* 100 micromolar) typically used in formulations of therapeutic proteins, thermodynamic effects due to excluded volume can usually be neglected. More important is specific binding of surfactants to either the native or unfolded states of a protein. Randolph and colleagues report that some proteins and nonionic surfactants, including Tween 20, form mixed protein: detergent complexes (Bam et al., 1995, 1996, 1998; Jones, et al., 1999). In the presence of Tween 40 at a 4:1 surfactant: protein molar ratio, native recombinant human growth hormone is significantly stabilized: the denaturation midpoint in guanidine hydrochloride solutions increases from 4.6 M guanidine hydrochloride in the absence of Tween 40 to 5.9 M in the presence of Tween 40 (Bam et al., 1996). Likewise, 4:1 Tween 40 increases the ΔG of unfolding of recombinant human growth hormone by 4.1 kcal/mol (Bam et al., 1996), and 10:1 Tween 40 increases its melting point slightly from 88.8 to 89.4° C (Bam et al., 1998). Stabilization of recombinant human growth hormone by surfactants results in reduced aggregation in agitated solutions (Bam et al., 1998). With bovine serum albumin in the presence of surfactant there are decreased amounts of thermally-induced protein aggregates, relative to surfactant-free controls (Arakawa and Kita, 2000). Conformational stabilization of proteins by non-ionic surfactants is not universal; stability of IgG is unaffected by low concentrations of Tween 20 (Vermeer and Norde, 2000), and recombinant human interferon-γ shows lower free energies of unfolding in the presence of Tween 20 (Webb et al., 2000).

SURFACTANT EFFECTS ON PROTEIN ASSEMBLY STATE

The hydrophobic portion of non-ionic surfactants can bind to hydrophobic patches on proteins. This naturally causes the surfactant to order itself so that more hydrophilic groups are solvent exposed, resulting in a "hydrophobicity

Apotex Exhibit 1027
Page 9 of 17

reversal". This "hydrophobicity reversal" means that the protein-surfactant complex is more hydrophilic that either the surfactant or protein alone, and effectively increases the solubility of the complex. This, in turn can reduce the propensity of the protein to form higher-order aggregates. For example, bovine mitochondrial cytochrome bc1 is dimeric in solutions at low ionic strength and low surfactant levels. At Tween 20 concentrations above 5 mg/mg protein, a homogeneous, monomeric, reversible and enzymatically active protein-surfactant complex is formed (Musatov and Robinson, 1994). Likewise, in freeze-thaw studies of recombinant factor XIII, addition of Tween 20 at concentrations near the CMC blocked the progression of aggregates from a relatively low molecular weight, soluble fraction to insoluble aggregates. Figure 4 shows levels of

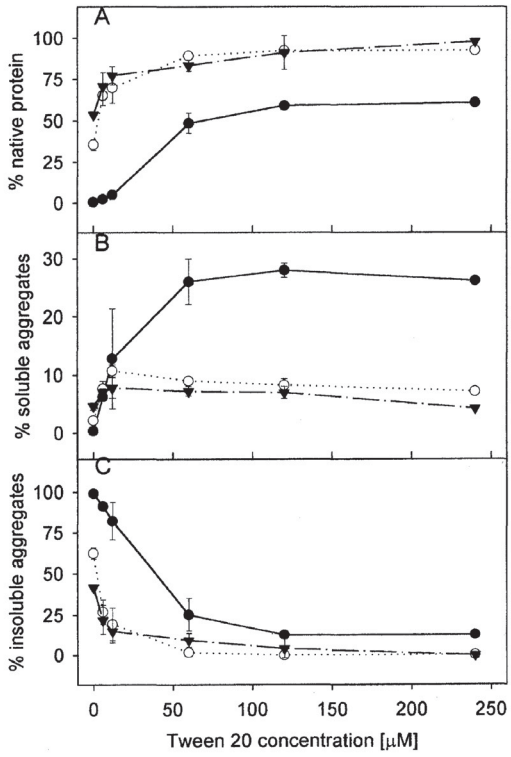


Figure 4. Recovery of native rFXIII (A) and formation of soluble (B) and insoluble aggregates (C) following 10 freeze-thaw cycles of 1 mg/ml (—●—), 5 mg/ml (·····○·····) and 10 mg/ml rFXIII (—▼—) as a function of Tween 20. Results are plotted as mean values +/– standard deviation for duplicate samples (reproduced from Kreilgaard et al., 1998).

Apotex Exhibit 1027
Page 10 of 17

aggregation for factor XIII after freeze thaw cycling in the presence and absence of Tween 20. Note that Tween addition did not completely block aggregation, but was very effective at preventing the formation of insoluble aggregates (Kreilgaard et al., 1998).

Non-ionic surfactants can also have the opposite effect on protein assembly state. In cases where a non-ionic surfactant destabilizes the conformation of a protein, this effect may compete against the solubilizing effect of surfactant binding and hydrophobicity reversal. For example, Bax, a monomeric protein that regulates apoptosis, readily forms dimers in the presence of Tween 20. However, these dimers are apparently non-native, as they do not expose the characteristic N-terminal Bax epitope (Hsu and Youle, 1998). In the case of the hydrophobic lipase from *Humicola lanuginose*, Tween 20 addition caused the formation of large, insoluble non-native aggregates (Kreilgaard et al., 1999).

SURFACTANT EFFECTS ON PROTEINS DURING FREEZING, FREEZE-DRYING AND RECONSTITUTION

The processes of freezing, drying, and reconstitution of protein solutions present a number of stresses that may denature proteins. Many of these stresses are associated with surfaces: new ice-water and ice-glassy solid interfaces are formed during freezing, drying replaces ice-glass interfaces with air-glass interfaces, and reconstitution exposes the glassy solid surfaces to aqueous solution. In each of these steps protein adsorption to surfaces is potentially damaging. The ice-water interface has been implicated as a source of damage to proteins (Strambini and Gabellieri, 1996), as has the solid-air interface (Hsu et al., 1995). Addition of nonionic surfactants can reduce this damage, presumably by competing with the protein for the ice-water interface (Chang, 1996). For example, addition of Tween 80 to solutions of recombinant hemoglobin reduced aggregation seen during freeze thaw studies (Kerwin et al., 1998). Interestingly, Tween 80 did not offer protection against methemoglobin formation or hemoglobin aggregation during long-term frozen storage.

Non-ionic surfactants also have been shown to affect the recovery of native protein from lyophilized formulations. Sarciaux et al. (1999) showed that the addition of Tween 80 to the formulation solution or the reconstitution medium for lyophilized formulations resulted in reduced levels of aggregates. Likewise, Zhang et al. (1995, 1996) have demonstrated that, following long-term storage, surfactants in the reconstitution medium can affect protein recovery. We have recently shown (Webb et al., 2000) that addition of Tween 20 to the reconstitution medium for lyophilized preparations of recombinant human interferon-γ results in decreased levels of aggregates. The mechanism for such reduced

Apotex Exhibit 1027
Page 11 of 17

aggregation was shown to be a surfactant effect on dissolution rates. Addition of Tween 20 slowed the dissolution of the lyophilized solid, allowing protein that partially unfolded during freeze-drying to refold before aggregating. Interestingly, Tween 20 in aqueous solutions destabilizes recombinant human interferon-γ against urea-induced unfolding, impedes refolding during rapid dilution from urea solutions, and actually increases aggregation during agitation.

ENZYMATIC DEGRADATION OF NON-IONIC SURFACTANTS

Although most non-ionic surfactants are thought of as chemically inert components of a formulation, specific chemical interactions between proteins can occur. For example, some enzymes show hydrolytic activity toward Tweens. Smegmatocin, an esterase from *Mycobacterium smegmatis*, shows a broad thermal and pH stability in its activity against Tween 80 (Tomioka, 1983). The byproduct of Tween 80 hydrolysis, oleic acid, is toxic to some bacteria. Similar bacteriocins, which require Tween their expression, have been ascribed to other mycobacteria (Saito et al., 1983). It is not clear how widespread esterase activity is against Tweens. However, it is clear that caution should be used when formulating proteins with esterase activities in Tween solutions.

RECOMMENDATIONS FOR PROTEIN FORMULATION

Clearly, it is desirable to minimize the addition of any excipient to a formulation. This rule of thumb is even more pertinent for surfactants, because there is ample evidence that high concentrations of surfactants can be destabilizing to protein structure. On the other hand, small amounts of surfactant often provide benefits in preventing aggregation that greatly outweigh any conformationally destabilizing effect. How then should surfactant levels be chosen for optimal formulation? The answer appears to depend on the mechanism(s) by which a particular protein is protected from damage by surfactant addition. In cases where surfactants act to stabilize the native state of a protein by binding to the protein, a specific surfactant:protein stoichiometry may need to be maintained in order to provide optimal protection. In these cases, changes in the protein concentration within a formulation will dictate proportional changes in surfactant concentration to maintain a fixed molar ratio. This appears to be the case for recombinant human growth hormone, where protection against agitation-induced damage correlated with the molar ratio of surfactant to protein rather than to the surfactant's CMC (Bam et al., 1998). In the case of specific binding, the choice of nonionic

Apotex Exhibit 1027
Page 12 of 17

surfactant may be important. In the case of recombinant human growth hormone, for example, different binding stoichiometries and degrees of protein stabilization were seen for a variety of common surfactants (Bam et al., 1995). A general recommendation for proteins that show specific binding to the native state of the protein is to formulate so that the ratio of surfactant to protein is slightly above the binding stoichiometry for a particular surfactant. The choice of surfactant may be dictated by the degree of stabilization (which should correlate with the degree of binding) provided to a protein by a particular surfactant.

In contrast, if no specific binding is seen, then maximum levels of protection generally correlate with the CMC of the surfactant. In this case, surfactant should be added at levels slightly above the CMC. The choice of surfactant is often dictated by a trade-off: surfactants with lower CMC's will require less surfactant in solution to saturate surfaces and reduce surface-induced damage to proteins. However, surfactants with low CMC's are much more difficult to remove from solution (e.g., by dialysis) if necessary, and also tend to be less soluble than surfactants with higher CMC's, raising the possibility of undesirable phase separation during processes such as freezing or lyophilization.

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Apotex Exhibit 1027
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Apotex Exhibit 1027
Page 14 of 17

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Apotex Exhibit 1027
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Apotex Exhibit 1027
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Apotex Exhibit 1027
Page 17 of 17