Albinterferon α_{2b} Adsorption to Silicone Oil–Water Interfaces: Effects on Protein Conformation, Aggregation, and Subvisible Particle Formation

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ABSTRACT: Silicone oil used as a lubricant in prefilled syringes has the potential to induce formation of particles in protein formulations. In the current study, we used a therapeutic fusion protein, albinterferon α_{2b}, to evaluate protein aggregation and particle formation in the presence of silicone oil microdroplets or immobilized silicone interfaces. Tertiary structure of albinterferon α_{2b} adsorbed on silicone oil microdroplets was perturbed in a formulation containing only buffer. In contrast, native-like tertiary structure was retained for albinterferon α_{2b} adsorbed on silicone oil microdroplets in 300 mM sodium chloride or 300 mM sucrose formulations. Agitation of albinterferon α_{2b} samples in the presence of silicone oil droplets or siliconized beads, respectively, caused albinterferon α_{2b} aggregation and subvisible particle formation in formulations containing buffer or 300 mM sucrose. Adsorption of albinterferon α_{2b} onto silicone oil was inhibited by addition of 0.01% (w/v) polysorbate 80, and this excipient prevented aggregation during agitation in the presence of silicone oil microdroplets. Aggregation was also reduced in the presence of 300 mM sodium chloride during agitation at least in part because of the increased conformational stability of the protein. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:427–436, 2014

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INTRODUCTION

Therapeutic proteins are increasingly formulated in prefilled syringes, largely for patient convenience and dosing compliance. Silicone oil is used to lubricate the barrel-plunger assembly[1,2] and is thought to contribute to the widely observed protein aggregation in prefilled syringes.[1,3,4] In several studies, the combination of silicone oil and agitation in the presence of air–water interfaces led to synergistic increases in protein aggregation.[1,3,5] In contrast, in the same studies and others, incubation of proteins in the presence of silicone oil without agitation caused minimal aggregation.[3,5–9]

It seems likely that adsorption of the protein to the silicone oil–water interface initiates the process by which silicone oil induces protein aggregation. The levels of adsorption for other proteins to this interface have been reported to be between 1.5 and 6.5 mg/m^2.[5,6,10,11] A few studies have shown that nonionic surfactants inhibit protein adsorption on silicone oil.[3,6,8,11] In one study with an IgG_1, diminished adsorption correlated with lower levels of protein aggregation during agitation in the presence of silicone oil.[3] However, it is not known whether the inhibition of adsorption of protein to the silicone oil–water interface is generally effective at inhibiting aggregation during agitation.

In two recent studies, both focusing on monoclonal antibodies, adsorption to silicone oil–water interfaces was accompanied by perturbations in the tertiary structure of the proteins.[5,11] Britt et al.[11] showed that three different humanized antibodies showed essentially the same degree of tertiary structure perturbation after adsorption to silicone oil–water interfaces as that observed after incubation in 8 M urea. In contrast, somewhat smaller perturbations of tertiary structure were observed for another antibody upon adsorption to silicone oil microdroplets, and the perturbations were diminished when the ionic strength of the formulation was increased.[5] Interestingly, agitation-induced aggregation of the antibody in the presence of silicone oil was also reduced at high ionic strength.[5]

The connections between protein adsorption on silicone oil microdroplets,[6] accompanying protein tertiary structural changes[11] and aggregation during agitation[3] have only been evaluated in a single study of a monoclonal antibody that did not investigate the effects of addition of surfactants.[5] We hypothesize that addition of nonionic surfactants to formulations will not only inhibit protein adsorption to the silicone oil–water interface and associated protein structural perturbations, but will also inhibit protein aggregation during agitation. Furthermore, we hypothesize that in surfactant-free formulations the use of solution conditions that reduce the degree of structural perturbation of protein adsorbed to the silicone oil–water interface will also reduce aggregation during agitation.

In the present study, we used albinterferon α_{2b} as a model protein. Albinterferon α_{2b} is a fusion protein composed of human serum albumin (HSA) and interferon α_{2b} (Ifn-α).[12] Four albinterferon α_{2b} formulations were evaluated in the current study. These formulations contained either buffer (10 mM sodium phosphate, pH 7.2), buffer with 300 mM sodium chloride, buffer with 300 mM sucrose, or buffer with 0.01% (w/v)

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polysorbate 80. In these formulations, we measured the adsorption of albinterferon α_{2b} to silicone oil microdroplets and assessed accompanying tertiary structural changes. In addition, we evaluated the effect of agitation on the aggregation of albinterferon α_{2b} in the presence and absence of low concentrations of silicone oil microdroplets (0.2 mg/mL). This concentration of silicone oil was used to approximate typical silicone oil concentrations found in commercially available prefilled syringes.[4]

Furthermore, in luer-lock prefilled syringes or prefilled cartridges, silicone oil may be present as an immobilized layer that is "baked-on" the interior glass surfaces of syringe barrels. In a previous study, we showed the utility of using siliconized glass beads as a model for baked-on silicone interfaces to assess their effects on protein aggregation and subvisible protein particles formation.[9] In the current study, we also use siliconized beads to assess the influence of baked-on silicone water interfaces and agitation on the formation of subvisible particles and aggregates in albinterferon α_{2b} formulations.

MATERIALS AND METHODS

Materials

Purified albinterferon α_{2b} was provided by GlaxoSmithKline (Rockville, Maryland) as a stock solution containing 5 mg/mL albinterferon α_{2b}, 36 mg/mL mannitol, and 23 mg/mL trehalose in 10 mM sodium phosphate buffer, pH 7.2. Reagent grade buffer salts were purchased from Fisher Scientific (Fair Lawn, New Jersey). Polysorbate 80 (product #4117-04) and endotoxin-free sucrose (product #4005-04) were obtained from J.T. Baker (Avantor Performance Materials Inc., Phillipsburg, New Jersey). Medical grade silicone oil (Dow Corning 360™, 1000 cSt) was purchased from Dow Corning Corporation® (Midland, Michigan). Hexane was purchased from Acros Organics™ (New Jersey). Pierce Slidealyzer® dialysis cassettes (20 kDa molecular weight cutoff; product #66012) were purchased from Thermo Scientific® (Rockford, Illinois). Sterile 3 cc type I glass vials were purchased from SCHOTT® (Elmsford, New York), and sterile vial stoppers (West 4432/50 13 mm serum stopper, USP type I) were obtained from West® Pharmaceutical Services (Lionville, Pennsylvania). Barrier tips (1 mL, catalog #BT1250) were purchased from Neptune® (San Diego, California). Nonporous (1 mm) borosilicate glass beads (catalog #Z273619) were obtained from Sigma–Aldrich (St. Louis, Missouri). The siliconizing agent Surfasil® was purchased from Thermo Scientific (Rockford, Illinois).

Methods

Albinterferon α_{2b} Sample Preparation

The buffer (10 mM sodium phosphate, pH 7.2) used throughout the study was prepared from deionized water, filtered through a 0.22 μm nitrocellulose membrane and used for dialysis of the stock protein solution. To prepare excipient-free formulations, albinterferon α_{2b} was dialyzed for twelve hours (4 °C) against buffer using dialysis cassettes. For formulations with excipients, albinterferon α_{2b} was dialyzed against buffer solutions containing either 300 mM sucrose or 300 mM sodium chloride. For the preparation of albinterferon α_{2b} formulations containing polysorbate 80, an aliquot of freshly prepared stock of 1% (w/v) polysorbate 80 in buffer was added to the dialyzed protein sample to achieve a final polysorbate 80 concentration of 0.01% (w/v). Albinterferon α_{2b} concentrations were determined from UV absorbance at 280 nm (Agilent® 8540 spectrophotometer, Santa Clara, California), using an extinction coefficient of 0.621 AU mL mg^{-1} cm^{-1} (provided by GlaxoSmithKline). The final albinterferon α_{2b} concentration was adjusted to 3 mg/mL by dilution of the dialyzed protein solution with the appropriate formulation.

Silicone Oil-in-Water Emulsions

Surfactant-free, silicone oil-in-water emulsions were prepared following a previously published method.[6] The resulting emulsion was collected in a clean glass bottle and stored at room temperature until use. Stock solutions of the excipients (2 M sodium chloride, 1 M sucrose, or 1% polysorbate 80) were prepared separately in buffer and added to an appropriate volume of the emulsion. The final concentrations of these excipients in the silicone oil emulsion were 300 mM sodium chloride, 300 mM sucrose, or 0.01% (w/v) polysorbate 80. Following the addition of excipients, the emulsions were swirled gently and allowed to stand for 10 min at room temperature before use.

The silicone oil concentration in the emulsion was determined following a previously published method wherein the silicone was first extracted into hexane[6] and then analyzed by infrared spectroscopy at 1220–1300 cm^{-1}.[13]

Estimation of Total Interfacial Area of Silicone Oil Microdroplets in Aqueous Emulsion

The size distribution of the silicone oil microdroplets in the aqueous emulsion was determined with a Beckman Coulter LS230 (Fullerton, California), using a refractive index of 1.405 for silicone oil.[14] The silicone oil microdroplet number distribution in the buffer-only formulation along with the concentration of silicone oil in the emulsion were used to estimate the total interfacial area of silicone oil microdroplets.[11] Consistent with observations reported by Ludwig et al.,[6] the addition of either 300 mM sucrose or 300 mM sodium chloride to the buffer-only silicone oil emulsion increased the droplet diameter slightly (data not reported) and, hence, reduced total interfacial surface area by about 33%. The droplet diameter in the 0.01% polysorbate 80 formulation was comparable to that in buffer-only formulation (data not reported).

Measurement of Albinterferon α_{2b} Adsorption onto Silicone Oil Microdroplets

A centrifugation method was used to measure albinterferon α_{2b} adsorption on silicone oil microdroplets in aqueous solutions.[6,11] First, 980 μL samples containing various concentrations of silicone oil emulsion were prepared by pipetting increasing volumes of stock silicone oil emulsion into a buffer solution in polypropylene Eppendorf tubes (Fisher Scientific Catalog #05-402-25). Then, 20 μL of 0.25 mg/mL albinterferon α_{2b} was added to achieve an albinterferon α_{2b} concentration of 5 μg/mL in the final sample. This method was used to prepare samples in buffer only, 300 mM sucrose, and 300 mM sodium chloride. For studies with polysorbate 80, to prevent preadsorption of the surfactant on silicone oil microdroplets prior to protein addition, samples for 0.01% (w/v) polysorbate 80 formulation containing 5 μg/mL albinterferon α_{2b} and silicone oil emulsion were prepared by a slight modification of the above method. First, 470 μL of buffer was pipetted into Eppendorf tubes. Then 10 μL