NOTE

Silicone Oil Induced Aggregation of Proteins

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ABSTRACT: Prior to delivery to the patient, protein pharmaceuticals often come in contact with a variety of surfaces (e.g., syringes and stoppers), which are treated to facilitate processing or to inhibit protein binding. One such coating, silicone oil, has previously been implicated in the induction of protein aggregation. We have investigated the propensity of model proteins to aggregate when silicone oil is present in solution and find significant induction of aggregation in four proteins of various molecular weights and isoelectric points in the presence of 0.5% oil. The ability of silicone oil to induce conformational changes that might be responsible for this aggregation was also examined by a combination of circular dichroism (CD) and derivative UV spectroscopy. Neither method produces evidence of large conformational changes or alterations in thermal stability although in a limited number of cases some small changes suggest the possibility of minor structural alterations. The most probable explanation for silicone oil induced aggregation is that the oil has direct effects on intermolecular interactions responsible for protein association through interaction with protein surfaces or indirectly through effects on the solvent. J Pharm Sci 94:918–927, 2005

Keywords: protein aggregation; UV-vis spectroscopy; silicone oil; circular dichroism

Silicone oil contamination has long been suspected of being responsible in some cases for the aggregation seen in certain protein pharmaceutical preparations. Several publications in the 1980s implicated the release of silicone oil from disposable plastic syringes in the aggregation of insulin.[1–5] The link between insulin aggregation and silicone oil was originally based on the observation that after multiple withdrawals from vials, patients using multi-dose preparations of insulin observed clouding of the solutions. In this regard, Chantelau et al. report a silicone oil contamination of up to 0.25 mg/mL in a 10 mL insulin vial when a standard procedure for filling 1 mL siliconized syringes was performed three times each using 10 syringes.[2] Referencing silicone oil contamination levels reported by a syringe manufacturer,[6] Bernstein calculated that some of his patients who were prescribed low doses of insulin could have vials containing ~4 mg of silicone oil when only 1/3 of the vial had been used.[4] The use of silicone oil is not limited to syringes. It is also used as a coating for porous glass vials to minimize protein adsorption and as a lubricant to prevent the conglomeration of rubber stoppers during filling procedures. In addition, it is the author's experience that questions of silicone oil contamination and its potential role in protein aggregation arise frequently during the pharmaceutical development of proteins generally, although little information about this potential

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problem is available in the scientific literature. Thus, the possibility that silicone oil induces the aggregation of proteins could have important implications for a wide variety of protein formulation and process development related phenomena.

Therefore, the purpose of this investigation was to assess the ability of silicone oil to induce aggregation of a variety of proteins over a range of pH and to investigate whether several biophysical techniques that are sensitive to changes in protein secondary and tertiary structure can detect silicone oil induced conformational changes that might be responsible for aggregation. Four model proteins (ribonuclease A (RNase A), lysozyme, bovine serum albumin (BSA), and concanavalin A (ConA)) with a wide range of different physical characteristics were used (Table 1). The choice of buffer pH was based on both pharmaceutical relevance and the well-characterized pH-dependent oligomerization state of ConA.[7] At the lowest pH examined (4.5), ConA is a dimer. At pH 6.5, it exists in dimeric and tetrameric forms. Above pH 7.0, ConA is primarily a tetramer.

EXPERIMENTAL

Materials

Chicken egg white lysozyme (L7651), bovine serum albumin (A3294), ConA from *Canavalia ensiformis* (C7275), and ribonuclease A from bovine pancreas (R5125) were purchased from Sigma Chemical Company (St. Louis, MO). These proteins can be lyophilized without a high content lyoprotectant. Thus, all proteins were supplied as essentially salt-free lyophilized powders and were used without further purification. Silicone oil (S159–500) was purchased from Fisher Chemical Company (Pittsburgh, PA). All buffer salts (sodium phosphate monobasic, sodium phosphate dibasic, sodium acetate, and sodium chloride) were ACS grade or higher. Solutions were prepared using distilled deionized water.

Methods

Preparation of Stock Solutions

Three buffers (10 mM sodium phosphate, 130 mM NaCl, pH 6.5 and pH 7.2 and 10 mM sodium acetate, 130 mM NaCl, pH 4.5) were used. A stock solution (suspension) of 1% (w/v) silicone oil in buffer was prepared by combining silicone oil and buffer in a 50 mL polypropylene centrifuge tube and sonicating for 10 min in an FS30 (Fisher Scientific) ultrasonicating bath to create a dispersion. All silicone oil suspensions were freshly prepared on the day they were used. Over the period of the experiments, the resulting dispersions were stable as judged by constant optical properties.

Protein solutions were prepared in each buffer by adding buffer to an appropriate amount of lyophilized protein to obtain a protein concentration between 1 and 2 mg/mL. The concentration of the protein was then determined based on its extinction coefficient, and additional buffer was added to adjust the protein concentration to 1 mg/mL. The proteins were kept on ice or refrigerated until used.

Turbidity

Optical density measurements were used to monitor protein aggregation. Equal volumes of protein and silicone oil stock solutions were combined in 1.7 mL microcentrifuge tubes to create concentrations of protein and silicone oil of 0.5 mg/mL and 0.5% (w/v), respectively. The samples were mixed by gentle pipetting and inspected visually to ensure a homogeneous appearance. Control protein samples in each buffer were

Table 1. Model Proteins

Protein	Molecular Weight and (pI)[12]	No. of Various Types of Secondary Structure Units		
		Helices	β-Sheets	Turns
Ribonuclease A (RNase A)	13.7 kDa (8.8)	4	12	13
Lysozyme	14.4 kDa (11.0)	4	5	11
Bovine serum albumin (BSA)	66 kDa (4.9)	60		
Concanavalin A (ConA)[b]	102 kDa (tetramer) (4.5–5.5)	5	26	

[a]Secondary structure content are based on the following structures deposited in the Protein Databank (RNase A: 4RAT; lysozyme: 4LYZ; BSA: 1AO6; ConA: 1APN). http://www.rcsb.org/pdb/

[b]The secondary structure content of ConA is given for a monomer unit.