Adsorption of Monoclonal Antibodies to Glass Microparticles

MATTHEW HOEHNE,[1] FAUNA SAMUEL,[1] AICHUN DONG,[1] CHRISTINE WURTH,[2] HANNS-CHRISTIAN MAHLER,[2] JOHN F. CARPENTER,[3] THEODORE W. RANDOLPH[1]

[1]Department of Chemical and Biological Engineering, University of Colorado, Boulder, Connecticut

[2]Formulation R&D Biologics, Pharmaceutical and Analytical R&D, F. Hoffmann-La Roche Ltd, Basel, Switzerland

[3]University of Colorado at Boulder, Denver, Connecticut

Received 11 January 2010; revised 29 March 2010; accepted 30 April 2010

Published online 23 June 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.22275

ABSTRACT: Microparticulate glass represents a potential contamination to protein formulations that may occur as a result of processing conditions or glass types. The effect of added microparticulate glass to formulations of three humanized antibodies was tested. Under the three formulation conditions tested, all three antibodies adsorbed irreversibly at near monolayer surface coverages to the glass microparticles. Analysis of the secondary structure of the adsorbed antibodies by infrared spectroscopy reveal only minor perturbations as a result of adsorption. Likewise, front-face fluorescence quenching measurements reflected minimal tertiary structural changes upon adsorption. In contrast to the minimal effects on protein structure, adsorption of protein to suspensions of glass microparticles induced significant colloidal destabilization and flocculation of the suspension. © 2010 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:123–132, 2011

Keywords: adsorption; biocompatibility; stability; proteins; protein formulation

INTRODUCTION

Liquid formulations of therapeutic proteins offer numerous advantages in terms of patient convenience and compatibility with delivery devices such as autoinjector systems. Although such formulations are becoming more common, their development can be hindered by the formation of protein aggregates, which can occur as a result of a number of stresses that proteins experience during manufacturing, storage, and shipping.[1–3]

Even after careful optimization of a liquid formulation so as to maximize the stability of a protein, minute populations of subvisible or visible particles may be found. These particles may originate from process equipment (e.g., metal particles from pumps used during fill-finish operation, from the packaging materials (e.g., glass particles from vial surfaces) or from the formulation itself (e.g., protein aggregates formed upon storage).

Within a formulation that provides adequate (e.g., 18–24 months shelf life) stability, the appearance of relatively few visible particles in only some vials of a given production lot cannot easily be explained by global instability of the protein. An alternative explanation could be that such particles arise from interactions of the protein with subvisible, trace amounts of foreign microparticles such as the glass that can in some cases delaminate from the inner surface of glass vials,[4] depending on glass processing and quality. However, it has also been suggested that such microparticulate contamination of the bulk solution is unavoidable regardless of the quality of glass.[5] There are a number of cases in which the interaction of a therapeutic protein with the surfaces of common materials such as those used in container closures or delivery devices caused protein aggregation.[6–9] Recently there has been an increasing emphasis on aggregation caused by microparticulate contaminants.[9–12]

Because their genesis is likely to be from the walls of the final containers, subvisible glass microparticles might be present in some final products. If they remain as subvisible particles, they would be difficult to detect and identify and unlikely to result in failures during container inspections. We hypothesize, however, that if such particles interact with proteins, for example, initiating protein aggregation through nucleation and growth mechanisms[13] or agglomerate to form larger particles, insoluble aggregates and larger, visible particles eventually may result.

Interactions of proteins with glass microparticles potentially could result in the production of larger visible particles via at least two pathways. Proteins are known to adsorb to a variety of interfaces,

Correspondence to: Theodore W. Randolph (Telephone: 303-492-4776; Fax: 303-492-4341; E-mail: theodore.randolph@colorado.edu)

Journal of Pharmaceutical Sciences, Vol. 100, 123–132 (2011)

including the glass-water interface.[14] The total glass surface area that would be present in a formulation would be expected to be so small that losses of soluble protein due to simple adsorption would be insignificant. However, adsorption of the protein to the surface of a microparticle might serve to nucleate aggregation if the adsorbed protein were to (partially) unfold, which could further react with protein in the bulk solution to form larger aggregates. Alternatively, protein adsorption to microparticulate contaminants could alter colloidal interactions between the microparticles themselves, resulting in colloidal destabilization and eventual flocculation of glass microparticles.

To test whether the addition of microscopic glass particles might induce aggregation or flocculation via either of these mechanisms, we investigated the interactions of three humanized monoclonal antibodies (humAbs) with glass microparticles. First, we measured the adsorption of the antibodies to glass microparticles and directly examined humAbs adsorbed onto the glass surface by atomic force microscopy (AFM). Changes in the secondary structure of the adsorbed proteins after adsorption to glass microparticle surfaces were probed by FTIR spectroscopy, and changes in tertiary structure were monitored in front-face fluorescence quenching studies. Changes in microparticle colloidal stability induced by adsorption of protein were followed by recording changes in macroscopic settling rates as microparticles agglomerated. In addition, we varied the solutions in which the proteins were formulated. For some experiments, NaCl (140 mM) was added to histidine-buffered solutions to increase charge electrostatic screening in order to probe the importance of electrostatic interactions in determining adsorption and flocculation behavior. In other experiments, sucrose (240 mM) was added to increase the conformational stability of the antibodies in order to test the importance of potential conformational changes.

MATERIALS AND METHODS

Materials

Three recombinant humanized monoclonal antibody (humAb1, humAb2, and humAb3) formulations were obtained from F. Hoffmann-LaRoche Ltd (Basel, Switzerland). humAb1 and humAb2 were provided as frozen liquid formulations of 89.1 and 10.26 mg/mL, respectively, and stored at −80°C. humAb3 was provided as a liquid formulation of 10.30 mg/mL and stored at 2–8°C. Additional properties for each protein are summarized in Table 1. For the studies presented here, the proteins were dialyzed into one of three buffers adjusted to pH 6.0 using HCl. The buffers were formulated as follows: 10 mM L-histidine, pH 6.0, 0.1 g/L NaN_3 (buffer 1) or 10 mM L-histidine, pH 6.0, 140 mM NaCl, 0.1 g/L NaN_3 (buffer 2), or 10 mM L-histidine, pH 6.0, 240 mM sucrose, 0.1 g/L NaN_3 (buffer 3). All chemicals not specifically mentioned were of reagent grade or higher quality. Sodium azide was contained in the formulations to avoid microbiological growth under testing conditions, but is not expected to have interfered with results obtained.

Table 1. Some Physical Properties of the Three humAbs

	Mol. Weight (g/mol)	ε_{280} (cm^2/mg)	p*I* (Calculated)
humAb1	146,243	1.4	9.0–10.0
humAb2	145,996	1.49	8.8–8.9
humAb3	152,942	1.57	9.3

Ground Glass and Silica Microparticles

Glass microparticles were prepared directly from type 1 glass vials (Vial, 6 mL, Fiolax Type, Schott AG, Müllheim, Germany) by ball-milling broken glass shards (<2 mm) with zirconium oxide ball-grinding media (Union Process, Akron, OH) and sieving with a 45 μm screen to form a stock powder preparation. All glass microparticles used in these experiments were from a single grinding lot. Silica microparticles were purchased from Sigma–Aldrich (St. Louis, MO).

Glass Microparticle Surface Area and Size Distribution

Particle-specific surface areas for glass and silica microparticles were determined using nitrogen adsorption and Brunauer–Emmett–Teller (BET) isotherm analysis using an Autosorb 1C (Quantachrome Instruments, Boynton Beach, FL). The relative pressure (P/P_o) range was from 0.05 to 0.3. The BET runs were conducted under isothermal conditions in a liquid nitrogen bath.

In addition, the size distribution of suspensions of glass microparticles in deionized water were measured in triplicate using a Coulter LS230 light scattering instrument (Beckman Coulter, Inc., Miami, FL) and reported as the mean surface area-weighted size.

Protein Assays

Soluble antibody levels were determined using HP-SEC on a TSK-GEL G3000SW_{XL} column and SW guard column (Tosoh Bioscience LLC, Montgomeryville, PA). HP-SEC was performed with a Beckman Coulter System Gold HPLC consisting of a 126 pump (Beckman Coulter, Inc., Fullerton, CA), a Waters 717 Plus autosampler (Waters Corp., Milford, MA), and a 166 UV detector (Beckman Coulter, Inc., CA). The mobile phase consisting of 0.2 M potassium phosphate, 0.25 M potassium chloride, and 0.01% (w/v) sodium azide (pH 7.0) was set at a flow rate of