Reversible Self-Association Increases the Viscosity of a Concentrated Monoclonal Antibody in Aqueous Solution

JUN LIU,[1] MARY D.H. NGUYEN,[1] JAMES D. ANDYA,[2] STEVEN J. SHIRE[1]

[1]Late Stage Pharmaceutical and Device Development, Genentech, Inc., 1 DNA Way, South San Francisco, California 94080

[2]Early Stage Pharmaceutical Development, Genentech, Inc., South San Francisco, California 94080

Received 22 July 2004; revised 9 February 2005; accepted 11 February 2005

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.20347

ABSTRACT: This study was conducted to investigate the effect of reversible protein self-association on the viscosity of concentrated monoclonal antibody solutions. The viscosities of the monoclonal antibody solutions were measured by either a capillary viscometer or a cone-plate rheometer at different protein concentrations, pH, and ionic strength. Soluble aggregates were determined by size exclusion chromatography, light scattering, and analytical ultracentrifugation. Self-association of protein at high protein concentration was monitored by sedimentation equilibrium analysis using a preparative ultracentrifuge and a microfractionator. The viscosity of one of the monoclonal antibodies investigated is highly dependent on protein concentration, pH, and ionic strength of buffer and charged excipients. This antibody shows the highest viscosity near its pI at low ionic strength conditions. Sedimentation equilibrium analysis suggests that this antibody tends to reversibly self-associate at high protein concentration. The self-association appears to be quite weak and is not detectable by sedimentation velocity and size exclusion chromatography at low protein concentration. There are no significant differences in the amounts of non-dissociable soluble aggregates formed between low viscosity and high viscosity samples. These results suggest that the reversible multivalent self-association of this protein appears to be mediated mainly by electrostatic interactions of charged residues and results in unusually high viscosity of this monoclonal antibody in solution at low ionic strength conditions. J Pharm Sci 94:1928–1940, 2005

Keywords: monoclonal antibody; high protein concentration; viscosity; self-association; sedimentation equilibrium; sedimentation velocity

INTRODUCTION

Over the last decade, monoclonal antibody therapy has evolved and become an important class of drugs for treating numerous human diseases, including cancer, allergic diseases, asthma, and organ transplantation.[1–3] Approximately 25% of protein drugs currently under development are full length, fragment, or conjugated versions of monoclonal antibodies (http://www.phrma.org). Since antibodies are part of the natural human defense systems that are capable of interacting with virtually any antigen molecules at high affinity and specificity, they are ideal candidates for targeted therapy. By the end of 2003, fourteen therapeutic antibodies, antibody fragments, or conjugated antibodies had been licensed by the US Food and Drug Administration (FDA) to treat a wide range of human diseases.[4]

Monoclonal antibody therapy is often given on a regular basis and requires several mg/kg dosing by injection.[5] The majority of approved monoclonal antibody therapeutics, especially for oncology indications is administered by the IV route.[6] However, several ongoing monoclonal antibody development

Correspondence to: Jun Liu (Telephone: 650-225-1248; Fax: 650-225-7234; E-mail: liu.jun@gene.com)

Journal of Pharmaceutical Sciences, Vol. 94, 1928–1940 (2005)

programs are targeting diseases that may require outpatient administration or home use, and hence may necessitate the development of alternate delivery routes. Oral, transdermal, and pulmonary routes of administration have been difficult to achieve because of the instability and sizes of monoclonal antibodies. For example, one monoclonal antibody given by the pulmonary route had low systemic distribution and was clinically ineffective.[7] Subcutaneous (SC) administration is the preferred route used in a physician's office/clinic or by the patient at home. SC administration is generally limited to small injection volume, and for the high dose antibody therapy, it often necessitates the development of high protein concentration formulations. For example, if a protein is to be administrated to a patient at 2 mg/kg on a weekly basis, the average weekly dose will be 130 mg considering 65 kg as the average body weight for patients. Since an injection volume of more than 1.5 mL is not well tolerated for SC administration, the protein concentration for weekly SC administration would have to be approximately 100 mg/mL (130 mg protein in less than 1.5 mL volume). Because protein molecules are large and complex, development of protein formulations at such high concentration poses many challenges. These include not only maintaining the physical and chemical stability throughout the shelf-life, but also having physical properties that are compatible with manufacturing, storage, and delivery.[8] Concentration dependent protein properties such as viscosity can have a major impact on the manufacturing process, especially tangential flow filtration (TFF) techniques used to concentrate and formulate the protein.[8] In particular, high viscosities may result in pressure drops during TFF that exceed the pump performance specifications. High viscosity of a protein formulation can also make it more difficult to administer the protein drug by injection, particularly for SC delivery, where delivery of high viscosity of solution within a reasonable time frame requires the use of larger bore needles, which may result in more painful SC injections. Therefore, it is important to optimize the formulation resulting in a reduced viscosity that is well suited for manufacturing and administration.

Most protein molecules are only marginally stable in solution. The structure and conformation of native protein can be altered under stressed conditions and lead to formation of irreversible soluble aggregates or particulates. Although molar concentration of osmolytes, such as sugars, have been used to prevent protein aggregation from nonnative protein in solution,[9,10] this apparent fix is limited since it may also add to the viscosity and osmolality of the formulation that may render it impractical for use as a SC delivery. In addition, high concentration of sugar can enhance the reversible self-association of native protein,[11–13] particularly at high protein concentration, in which the weak reversible self-association can increase significantly. This can have a major impact on the physical properties of protein formulations. Due to these challenges, to date, very few marketed therapeutic monoclonal antibodies have been successfully formulated as liquids above 100 mg/mL.

Reversible self-association of a human myeloma protein has been shown to increase viscosity through a combination of weak nonionic or hydrophobic interactions.[14] In addition, self-association of monoclonal serum immunoglobulins has also been associated with the hyperviscosity syndromes of various disorders.[15–17] The present study seeks to understand the effects and contributions of a reversible protein self-association on the viscosity of a concentrated monoclonal antibody solution. These studies led to the development of an improved formulation with reduced viscosity that was well suited for SC delivery.

MATERIALS AND METHODS

Materials

Three humanized monoclonal antibodies with different complementary determining regions (CDRs), MAb1, 2, and 3 were constructed from the same IgG1 human framework with κ light chain. These antibodies were expressed in Chinese hamster ovary (CHO) cell lines, and prepared by a series of chromatography methods including affinity purification by protein A chromatography and ion exchange chromatography. The antibodies were prepared as lyophilized materials or as solutions using TFF processes. All the chemical reagents were analytical grade or higher. The actual compositions of the solutions are discussed in the appropriate "Results" section.

Analytical Methods

Size Exclusion Chromatography

Size exclusion chromatography experiments were conducted using a TosoHaas TSK Super SW 3000