Feasibility of Antibody–Poly(Glutamic Acid) Complexes: Preparation of High-Concentration Antibody Formulations and Their Pharmaceutical Properties

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ABSTRACT: Development of high-concentration antibody formulations for subcutaneous administration remains challenging. Recently, a precipitation–redissolution method was proposed to prepare suspensions or precipitates of salt-dissociable protein–poly(amino acid) complexes. To elucidate the utility of this method for protein therapy, we investigated the feasibility of a precipitation–redissolution method using poly(amino acid) for high-concentration antibody formulation. Omalizumab and adalimumab formulations of 150 mg/mL could be prepared using poly-L-glutamic acid (polyE) from low-concentration stock solutions. Enzyme-linked immunosorbent assay, circular dichroism, and size-exclusion chromatography revealed that the formation of antibody–polyE complex and precipitation–redissolution process did not significantly affect the immunoreactivity or secondary structure of the antibodies. The precipitation–redissolution method was less time-consuming and more effective than lyophilization–redissolution, evaporation–redissolution, and ultrafiltration from the viewpoint of final yield. Scalability was confirmed from 400 μL to 1.0 L. The general toxicity and pharmacokinetic profiles of the antibody–polyE complex formulations were similar to those of conventional antibody formulations. These results suggested that the precipitation–redissolution method using poly(amino acid) has great potential as a concentration method for antibody formulation and medicinal use.
Keywords: complexation; dissolution; polyelectrolytes; precipitation; formulation; suspensions

INTRODUCTION

Monoclonal antibodies have generated considerable interest as biopharmaceuticals over the last two decades because of their high target specificity and biocompatibility. At present, over 20 types of monoclonal antibody have been approved by the US Food and Drug Administration[1] and used for several types of disease, such as cancer and autoimmune diseases.[2–5] Despite advances in antibody drug development, the routes of administration for these antibodies remain challenging. The major route of delivery for antibodies has been intravenous administration because of the high bioavailability.[6] In contrast, subcutaneous administration has been in great demand as an alternative route that allows at-home administration by patients and improves compliance rates.[7–10] However, the desirable concentration of antibodies for subcutaneous administration is usually above 100 mg/mL with a volume limitation of 1.5 mL.[9,10]

Several methods have been developed to obtain high-concentration protein solutions that would enable proteins to be used in formulations, for example, with additives such as arginine or other amino acids,[11,12] ultrafiltration,[13] gelation,[14] crystallization,[15] liquid–liquid phase separation,[16] and spray drying.[6,17] However, these methods are still time-consuming and costly. Methods for the suspension or precipitation of protein have also been reported for use in concentrated protein formulations.[18,19] If the precipitates of protein can be fully resolubilized by the simple method, such precipitates can be used as concentrated protein solutions. Recently, we have demonstrated the complex precipitation–redissolution method with poly(amino acid) as a precipitant.[20] Briefly, charged polyelectrolytes, including poly-L-lysine and poly-L-glutamic acid (polyE), interact strongly with complementary charged proteins through multiple electrostatic interactions, resulting in the formation of a protein–polyelectrolyte complex (PPC),[20–26] which can often be precipitated depending on the experimental conditions, such as pH, ionic strength, and stoichiometric ratio.[24,27,28] PPC precipitates are then redissolved by the addition of buffer with high ionic strength such that the final concentration reaches 150 mM, which corresponds to physiological conditions.[20,28,29] This simple system has been applied successfully for several types of therapeutic protein, including enzymes, antibodies, and peptide hormones.[20] However, the utility of this precipitation–redissolution method for protein therapy is still unclear.

In this study, we demonstrated the feasibility of the complex precipitation–redissolution method using poly(amino acid) for high-concentration antibody formulation. As

Abbreviations used: CD, circular dichroism; ELISA, enzyme-linked immunosorbent assay; FcεRIα, high-affinity immunoglobulin epsilon receptor subunit alpha; HRP, horseradish peroxidase; IgE, immunoglobulin E; IgG, immunoglobulin G; MOPS, 3-(*N*-morpholino)propanesulfonic acid; p*I*, Isoelectric point; polyE, poly-L-glutamic acid; PPC, protein–polyelectrolyte complex; SEC, size-exclusion chromatography; TMB, 3,3′,5,5′-tetramethylbenzidine; TNF-α, tumor necrosis factor α; Tris, Tris(hydroxymethyl)aminomethane.

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described below, antibody formulations with concentrations over 100 mg/mL of adalimumab for autoimmune disease and omalizumab for allergic asthma could be prepared by addition of polyE. The formation of antibody−polyE complex and precipitation–redissolution processes did not significantly change the immunoactivity or secondary structure of the antibodies, and did not result in undesirable aggregation. Comparison of time required, yield, and aggregate ratio indicated that the precipitation–redissolution method was more effective for application than the conventional concentration methods, including lyophilization–redissolution, evaporation–redissolution, and ultrafiltration. The precipitation–redissolution method was successfully performed from a scale of 400 μL to 1.0 L, indicating that this method could be scaled up to 2500-fold. Finally, the general toxicity and pharmacokinetic profiles of the antibody–polyE complex formulation were similar to those of conventional antibody formulations. These results suggested that this simple method represents a new strategy for preparing high-concentration antibody formulations, and we expect that this method and complex formulations would be applicable for medicinal use.

EXPERIMENTAL

Materials

Adalimumab was obtained from transfected Chinese hamster ovary cell cultures and purified on a protein-A column. Omalizumab was purchased from Novartis Pharma KK (Tokyo, Japan) and purified on a protein-A column to remove the additives. Citrate, sodium chloride (NaCl), sodium phosphate, and potassium chloride (KCl) were purchased from Kanto Chemical Company, Inc. (Tokyo, Japan). Polysorbate 20 was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 3-(*N*-morpholino)propanesulfonic acid (MOPS) and Blocking One were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). Tris(hydroxymethyl)aminomethane (Tris) was obtained from Bio-Rad Laboratories (Hercules, California). Biotinylated antihuman immunoglobulin G (IgG) monoclonal antibody, rat IgG, and polyE sodium salt with average molecular weights of 3000–15,000 Da (polyE1) and 50,000–100,000 Da (polyE2) were obtained from Sigma Chemical Company (St. Louis, Missouri). Human immunoglobulin E (IgE) was obtained from Abcam (Cambridge, Massachusetts). Biotinylated antihuman IgE monoclonal antibody was purchased from Miltenyi Biotec GmbH (Bergisch Gladbach, Germany). Human tumor necrosis factor α (TNF-α) was obtained from Gibco Life Technologies Ltd. (Grand Island, New York). Human high-affinity immunoglobulin epsilon receptor subunit alpha (FcεRIα) was purchased from Sino Biological Inc. (Beijing, China). Streptavidin-labeled horseradish peroxidase (Avidin-HRP) was obtained from Thermo Fisher Scientific Inc. (Waltham, Massachusetts). 3,3′,5,5′-Tetramethylbenzidine (TMB) was obtained from KPL (Gennep, The Netherlands). Human IgG1 Therapeutic EIA Kit was purchased from Cayman Chemical Company (Ann Arbor, Michigan). Sucrose was obtained from Dai-Nippon Meiji Sugar Company, Ltd. (Tokyo, Japan). L-Histidine hydrochloride was purchased from Mitsubishi Tanabe Pharma Company (Osaka, Japan). L-Histidine was obtained from Kyowa Hakko Bio Company, Ltd. (Tokyo, Japan). Glucose 50% injection was purchased from Terumo Company (Tokyo Japan). These chemicals were of high-quality analytical grade and were used as received.

Preparation of Antibody–Poly(Amino Acid) Complex and Redissolution

The procedure of precipitation and redissolution of antibody−polyE1 complex was described as follows. *Step 1*: The antibody stock solutions containing low (1.0 mg/mL) and high (30 mg/mL) concentration antibodies in 10 mM buffer (MOPS at pH 6.5, for adalimumab; citrate at pH 5.5 for omalizumab) were prepared. *Step 2*: Aliquots of 200 μL of various concentrations of polyE1 in 10 mM buffer (MOPS at pH 6.5, for adalimumab; citrate at pH 5.5 for omalizumab) were mixed with aliquots of 200 μL antibody stock solution in the same buffer. *Step 3*: The samples of antibody−polyE mixture were centrifuged at 1000*g* for 3 min at 25°C. *Step 4*: The supernatant of 370 μL was removed and precipitates were resuspended by vortexing. The nominal value of the samples was 30 μL. *Step 5*: 10 μL of 600 mM NaCl in 10 mM buffer was added. The concentrations of antibodies for each step were determined from the absorbance at 280 nm using a spectrometer (SpectraMax Plus384; Molecular Devices Company, Ltd., Sunnyvale, California). The immnoreactivity, secondary structure, and aggregation ratio were determined as described below.

In addition to the above original scale experiments (400 μL), both 50-fold scale up (20 mL; medium scale) and 2500-fold scale up (1.0 L; large scale) experiments were performed. Table 3 shows the volumes of these scales at *Step 2*. A concentration of 0.2 mg/mL polyE1 solution was mixed with 2.0 mg/mL adalimumab solution, and then precipitation–redissolution procedures were performed as described above. It is to note that the final concentration of antibody at *Step 5* is five times higher than that at *Step 1*. The containers and centrifuges used for each scale were 1.5 mL centrifuge tubes (MS-4215M; Sumitomo Bakelite Company, Ltd., Tokyo, Japan) and Kubota 3740 for mini-scale, 50 mL centrifuge tubes (430829; Corning Inc., Corning, New York) and Kubota 5220 for medium-scale, and 1 L Nalgene Polycarbonate Centrifuge Bottles (3122-1010; Thermo Fisher Scientific Inc.) and Kubota 9900 for large scale. All centrifuges were purchased from Kubota Company (Tokyo, Japan).

Concentration of Antibody Solutions by Several Methods

To compare the efficiencies of the concentration methods, 150 mg/mL omalizumab and adalimumab solutions were prepared by the following methods. (1) *Precipitation–redissolution*: 250 μL of 0.15 mg/mL polyE1 in 10 mM buffer (MOPS, pH 6.5, for adalimumab; citrate, pH 5.5 for omalizumab) and 250 μL of 30 mg/mL antibodies in same buffer. The samples of antibody−polyE mixture were centrifuged at 1000*g* for 3 min at 25°C. Supernatant (462.5 μL) was removed and 12.5 μL of 600 mM NaCl in 10 mM buffer was added. The final volume of the concentrated antibodies was 50 μL. (2) *Lyophilization−redissolution*: 250 μL of 30 mg/mL antibodies in 10 mM buffer were added to vials and frozen onto a precooled shelf (Drying Chamber DRC-1100; Tokyo Rikakikai Company, Ltd., Tokyo, Japan) at −40 °C for 2 h. Subsequently, the frozen samples were primary dried at −20°C for 12 h, and were secondary dried at 25°C for 4 h under 0.67 Pa. After lyophylization, 50 μL of 10 mM buffer were added to the vials to redissolve the lyophilized cakes. (3) *Evaporation−redissolution*: 250 μL

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