BIOTECHNOLOGY

High Concentration Formulations of Recombinant Human Interleukin-1 Receptor Antagonist: II. Aggregation Kinetics

JOHN R. ALFORD,[1] BRENT S. KENDRICK,[2] JOHN F. CARPENTER,[3] THEODORE W. RANDOLPH[1]

[1]Department of Chemical and Biological Engineering, University of Colorado, Boulder, Colorado 80309

[2]Amgen Inc., Longmont, Colorado 80503

[3]Department of Pharmaceutical Science, School of Pharmacy, University of Colorado Health Sciences Center, Denver, Colorado 80262

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ABSTRACT: At high protein concentrations (i.e., 50–100 mg/mL) and 37°C, low solution ionic strength accelerates aggregation of recombinant human interleukin-1 receptor antagonist (rhIL-1ra). We have used a variety of physical and spectroscopic techniques to explain this observation. A population balance model was applied to a continuous mixed suspension, mixed product removal (MSMPR) reactor at steady-state to determine aggregate nucleation and growth rates. Nucleation rates increase at low ionic strength, while growth rates are unaffected. At low rhIL-1ra concentrations (i.e., <1 mg/mL), no conformational changes or differences in free energies of unfolding (ΔG_{unf}) were observed at 37°C over the solution ionic strength range of 0.025–0.184 molal used for aggregation studies. However, increasing the protein concentration to 100 mg/mL shifts the rhIL-1ra monomer–dimer equilibrium significantly at low ionic strength to favor dimerization, which is reflected in subtle conformational changes in the circular dichroism and second-derivative FTIR spectra. In addition to a reversible dimer, an irreversible dimer forms by second-order kinetics during incubation at 37°C. This noncovalent dimer does not significantly participate in further aggregation. The loss of native protein due to aggregation at 37°C was third order in protein thermodynamic activity due to the rate-limiting formation of an aggregation-prone trimer. This trimer forms from irreversible attractive monomer–reversible dimer interactions, which were quantified using second osmotic cross virial coefficients. Lastly, the activity coefficient of rhIL-1ra estimated from aggregation rates is 50% higher at 100 mg/mL protein concentration than at 50 mg/mL, in close agreement with predictions from a hard-sphere model for activity coefficients. J Pharm Sci 97:3005–3021, 2008

Keywords: protein aggregation kinetics; IL-1ra; ionic strength; monomer–dimer equilibrium; high protein concentration; activity coefficient; nucleation rate; growth rate; MSMPR; second osmotic cross virial coefficient

INTRODUCTION

Therapeutic protein formulations must prevent degradation of the native state during long-term storage.[1] The principal degradation pathway for

Correspondence to: Theodore W. Randolph (Telephone 303-492-4776; Fax: 303-492-4341;
E-mail: Theodore.Randolph@Colorado.Edu)

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many proteins is irreversible aggregation,[2,3] which may cause immunogenicity in patients.[4] High protein concentrations present unique challenges to formulation, especially with regard to increasing the propensity for aggregation.[5] In addition to the obvious concentration dependence of aggregation rate laws that are greater than zero order in protein concentration, high protein concentrations also drastically increase the protein thermodynamic activity coefficient.[6] For example, the activity coefficient of sickle hemoglobin approaches 10^3 at the solubility limit.[7] This nonlinear increase in the protein activity coefficient may lead to rates of aggregation that greatly exceed those expected based on dilute solution behavior. For example, hard-sphere models of activity coefficients[6] predict that, at a concentration of 125 mg/mL protein, a 20% increase in protein concentration to 150 mg/mL is expected to increase the thermodynamic activity by about 80%, which in turn might be expected to cause dramatic increases in aggregation rates.

Currently, aggregation studies conducted over short time periods at elevated temperatures are used to screen empirically for stable formulations.[8] Real-time stability tests are then performed on promising candidate formulations at storage conditions for the actual labeled storage time of the protein. Although still in their infancy, various modeling approaches are being developed to allow for better interpretation of accelerated stability studies and rational design of protein formulations. One such model that has been applied to protein aggregation is the Lumry–Eyring two state model.[9–11] The first step in this model is a reversible conformation change of the native protein into an aggregation-prone transition state (corresponding to the protein thermodynamic stability, ΔG_{unf}), followed by an irreversible assembly process (corresponding to the solution colloidal stability, which is related to B_{22}, the second osmotic virial coefficient). Either of these steps may be rate-limiting. As Chi et al.[12] demonstrated, recombinant human granulocyte stimulating factor (rhGCSF) can be stabilized against aggregation by maximizing the thermodynamic and colloidal stability. These physical parameters offer a promising approach for designing formulations to inhibit aggregation, although their importance needs to be verified for more proteins.

To test the importance of conformational and colloidal stability as parameters controlling aggregation under conditions where high protein concentrations would be expected to add significant nonideality, we conducted studies using recombinant human interleukin-1 receptor antagonist (rhIL-1ra), a 17 kDa cytokine inhibitor. Specifically, we examined the effect of buffer type and solution ionic strength (I) on rhIL-1ra aggregation at protein concentrations of 50 and 100 mg/mL, and determined reaction orders and appropriate rate constants. Based on the studies we proposed an assembly pathway for rhIL-1ra aggregation under accelerated temperature conditions (note that the conditions used in this study are not the same as the commercial formulation and storage conditions). Additionally, we used aggregate size distribution measurements in combination with a continuously operated protein precipitator to measure aggregation nucleation and growth rates as a function of ionic strength. We then sought to explain these observations in the context of a modified Lumry–Eyring model for aggregation that incorporates the proposed assembly mechanism for aggregation of rhIL-1ra combined with a hard-sphere model to account for volume-exclusion-based solution nonidealities found at high protein concentrations.

MATERIALS AND METHODS

Materials

Pharmaceutical grade rhIL-1ra was manufactured and purified (>99%) at Amgen Inc. The stock formulation contained 220 mg/mL protein concentration in citrate buffered saline (CSE): 10 mM sodium citrate buffer pH 6.5, 140 mM sodium chloride, and 0.5 mM ethylenediaminetetraacetic acid (EDTA). rhIL-1ra stock solution was maintained at −80°C until needed. All other chemicals were purchased from Amgen Inc. (Thousand Oaks, CA), Sigma Chemical Company (St. Louis, MO), Fisher Scientific (Pittsburg, PA) and were of reagent grade or higher quality.

Dialysis and Sample Preparation

Dialysis for buffer exchange was performed against excess solvent overnight using Pierce 10000 MWCO Slide-A-Lyzer Dialysis Cassettes or Pierce 10000 MWCO Snakeskin. The final protein concentration was approximately 100 mg/mL and it was assumed all EDTA was removed during dialysis. The calculated solution ionic strength for citrate and phosphate buffered solutions was adjusted for the Donnan effect as described in