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A Micro–Polyethylene Glycol Precipitation Assay as a Relative Solubility Screening Tool for Monoclonal Antibody Design and Formulation Development

Vishal M. Toprani [1], Sangeeta B. Joshi [1], Lisa A. Kueltzo [2], Richard M. Schwartz [2], C. Russell Middaugh [1], David B. Volkin [1, *]

[1] *Department of Pharmaceutical Chemistry, Macromolecule and Vaccine Stabilization Center, University of Kansas, 2030 Becker Drive, Lawrence, Kansas 66047*

[2] *Vaccine Production Program, Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892*

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ABSTRACT

Adequate protein solubility is an important prerequisite for development, manufacture, and administration of biotherapeutic drug candidates, especially for high-concentration protein formulations. A previously established method for determining the relative apparent solubility (thermodynamic activity) of proteins using polyethylene glycol (PEG) precipitation is adapted for screening and comparing monoclonal antibody (mAb) candidates where only limited quantities ($\leq$1 mg) are available. This micro-PEG assay is used to evaluate various broadly neutralizing mAb candidates to HIV-1 viral spike (gp120 and gp41 glycoproteins). Using ~1 mg of VRC01-WT mAb per assay, the precision of the micro-PEG assay was established. A series of 7 different broadly neutralizing mAbs to the HIV-1 viral spike proteins were compared by curve shape (%PEG vs. protein concentration), $\%PEG_{midpoint}$ determinations, and extrapolated apparent solubility values. Numerous formulation conditions were then evaluated for their relative effects on the VRC01-WT mAb. The PEG_{midpt} and apparent solubility values of VRC01-WT mAb decreased as the solution pH increased and increased as NaCl and arginine were added. A final optimization of the micro-PEG assay established that amounts as low as 0.1-0.2 mg can be used. Thus, the micro-PEG assay has significant potential as a relative solubility screening tool during candidate selection and early formulation development.

Introduction

Protein solubility is a key physical parameter not only for chemistry, manufacturing, and control bulk development activities but also for formulation development including long-term stability and facile administration of protein-based drugs.[1-3] In particular, monoclonal antibodies (mAbs) have emerged as a key class of protein drugs, administered either intravenously by medical professionals or subcutaneously by patients.[4] The latter typically requires the development of high protein concentration, stable liquid formulations that can deliver >100 mg per 1-mL injection.[3] In addition, as part of biopharmaceutical lead identification, *in vitro* and *in vivo* solubility is considered a critical "drug-like" property. Solubility measurements can be used not only to identify potential protein drug candidates but also to rank order them in terms of potential manufacturing, storage, or *in vivo* potency issues.[5]

There are many methods available for determining protein solubility including ultrafiltration, dialysis and concentration, or lyophilization and reconstitution.[2,6] These measurements, however, are often not practical or easily attainable because of either excessive amounts of protein required (several hundred milligrams) or experimental difficulties such as gel formation and aggregation.[1] An alternative approach to measure apparent protein solubility (apparent thermodynamic activity) is by addition of polyethylene glycol (PEG) that precipitates the protein primarily by exclusion volume effects.[7-9] As described in detail elsewhere, a

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* *Correspondence to:* David B. Volkin (Telephone: +785-864-6262; Fax: +785-864-5736).

E-mail address: volkin@ku.edu (D.B. Volkin).

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linear relationship between the log of protein concentration versus the weight percent PEG in solution, under certain conditions, allows calculation of the apparent solubility (i.e., thermodynamic activity) of proteins by extrapolation of experimental results to zero PEG concentration.[7,8] It should be noted that this extrapolated activity value may contain thermodynamic nonideality terms, and hence, the measured solubility is referred to as apparent protein solubility. PEG precipitation method has been successfully used to determine the apparent solubility of multiple proteins[7,10-14] and also as a formulation screening tool.[15] Recently, Gibson et al.[15] have used a high-throughput version of the PEG precipitation method to determine the apparent solubility and PEG_{midpt} values for an IgG1 mAb produced from different cell lines and formulated in different pH and buffer solutions. However, this methodology still requires relatively large amounts of protein (5-10 mg per sample per experimental condition) which may not be available during early stages of drug development.

In the past decade, in addition to the current antiretroviral drugs, new therapeutics are being developed to reduce and cure HIV-1 infections.[16] Broadly neutralizing mAbs against HIV-1 are considered as potential candidates for the development of a passive HIV-1 vaccine and have shown promise in the prevention and treatment of HIV-1 infection.[16-23] These broadly neutralizing mAbs offer several advantages over the current antiretroviral drugs such as viral neutralization and cell killing of the HIV-infected cells by Fc receptor-mediated effector mechanisms.[16] A major subset of these antibodies designated as the VRC01 class target the CD4-binding site on the gp-120 core of the virus and neutralize ~90% of diverse strains of the HIV virus.[16,22-24] Apart from the VRC01 class, there are several other categories of mAbs currently under development including the 10E8 and PGT classes which target the membrane proximal external region on gp41 and recognize epitopes such as the N-linked glycan at Asn 332 on gp120, respectively.[22,24] The low solubility of most of these mAbs, however, remains a key challenge during their development as candidates for a HIV therapy.[25,26]

In this work, we have developed a micro-PEG precipitation method requiring between 0.1 and 1.0 mg of protein using the VRC01-WT mAb. The relative apparent solubility profile of VRC01-WT mAb was established as measured by a combination of the curve shape (PEG vs. protein concentrations), PEG_{midpt} determinations, and extrapolated apparent solubility values. The micro-PEG method was then used as relative solubility screening tool for a developability assessment of small amounts of multiple mAbs being evaluated as possible neutralizing mAb candidates for HIV-1. We also demonstrate the utility of the micro-PEG method as an early formulation screening tool to evaluate various solution conditions (pH, buffers, salt concentration, and excipients) for their effect on the solubility profile of the VRC01-WT mAb.

Materials and Methods

Materials

A total of 7 mAbs (10E8-WT, 10E8v5, PGT121, 10E8v4, 35O22, VRC13, and VRC01-WT)[19,27-29] were provided by the National Institutes of Allergy and Infectious Disease (National Institutes of Health) in phosphate-buffered saline (PBS) at pH 7.4, except for VRC01-WT which was formulated in 25 mM sodium citrate, 50-mM sodium chloride, and 150 mM L-arginine hydrochloride at pH 5.8. The PGT antibodies were originally identified by the International AIDS Vaccine Initiative and published (Walker et al.[29]). Protein concentrations were determined by ultraviolet (UV) spectroscopy at 280 nm with an extinction coefficient of $\varepsilon^{0.1\%} = 1.6$ $(g/100\ mL)^{-1}$ cm^{-1}. All formulation reagents (sodium phosphate, sodium chloride, citric acid, sodium citrate, L-histidine, arginine, and PEG-10,000) for preparing different buffer systems, namely phosphate, citrate, and histidine, were purchased from Sigma-Aldrich (St. Louis, MO) and were of high-purity grade (>99%).

Methods

The protocol of the micro-PEG assay for assessing relative apparent solubility of mAbs was adapted from Gibson et al.[15] Stock solutions of PBS, pH 7.4, and PBS containing 40% w/v PEG-10,000 at pH 7.4 were mixed to prepare various concentrations of PEG solutions ranging from 0% to 40% w/v PEG. A volume of 200 μL of 22 different PEG-10,000 solutions was added in triplicate to wells of a 96-well polystyrene filter plate (Corning #3504; Corning Life Sciences, Corning, NY). The stock solution of VRC01-WT was diluted to 1 mg/mL with PBS buffer, pH 7.4. Fifty microliters of the protein stock solution (1 mg/mL) was then added to each well to a final protein concentration of 0.2 mg/mL. The plates were incubated overnight at room temperature and then centrifuged at 1233× *g* for 15 min; the filtrate was then collected in a clear 96-well collection plate (Greiner Bio-One#655001; Greiner Bio-One North America Inc., Monroe, NC). Thereafter, 200 μL of filtrate was transferred into a 96-well UV Star microplate (Grenier#655801). The filtrate was measured on a SpectraMax M5 UV-Visible plate reader at 280 nm to determine the protein concentration based on the extinction coefficient of $\varepsilon^{0.1\%} = 1.6$ $(g/100\ mL)^{-1}$ cm^{-1} (provided by Vaccine Research Center). This assay is referred to as the standard PEG precipitation assay in this article.

To further scale down the standard PEG assay, a NanoDrop spectrophotometer (NanoDrop Products, Wilmington, DE) was used as the UV detector for measuring protein concentration. The same procedure was followed for determining the protein concentration as described for the plate reader method, but with lower amounts and smaller volumes of protein. Forty microliters of various PEG-10,000 solutions were added to wells of a 96-well polystyrene filter plate, and 10 μL of the resulting protein solution (1 mg/mL) was added to the wells containing various levels of PEG to a final protein concentration of 0.2 mg/mL. Two microliters of the filtrate was measured with the NanoDrop spectrometer at 280 nm to determine the protein concentration using an extinction coefficient of 16 at 280 nm for a 1% (10 mg/mL) mAb solution. This version of PEG assay is referred to as the micro-PEG assay.

The micro-PEG assay was used to evaluate the relative solubility profiles of various mAbs that target the HIV-1 viral spike (gp120 envelope glycoprotein and gp41 transmembrane glycoprotein) and to compare the relative solubility profiles of VRC01-WT under different formulation conditions (pH, buffer, salt, and arginine). For the additional scaled-down version of the micro-PEG assay using 0.1-0.2 mg of protein, experiments with VRC01-WT containing different concentrations of arginine (0, 17, 50, and 150 mM) in 50 mM citrate buffer, pH 6.0, were first performed using 10 different %PEG concentrations, followed by repeating at 0% and 8.3% w/v PEG-10,000 for 5 replicates and calculating the %VRC01-WT soluble at 8.3% w/v PEG-10,000.

The absorbance at 280 nm (protein concentration) versus PEG-10,000 (%w/v) data were fit to a standard 4-parameter, modified Hill-slope sigmoidal curve equation (Eq. 1 as described in Gibson et al.),[15] using Python (x,y) version 2.7.6.0, an open-source scientific and engineering software based on the python language. The $\%PEG_{midpt}$ (*x*-axis midpoint) was calculated from the resulting curve fit using Equation 1, and the apparent solubility was calculated by fitting the data points from the transition region (i.e.,