Technical Decision Making with Higher Order Structure Data: Impact of a Formulation Change on the Higher Order Structure and Stability of a mAb

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ABSTRACT: Changes in formulation may be required during the development of protein therapeutics. Some of the changes may alter the protein higher order structure (HOS). In this note, we show how the change from a trehalose-based formulation to an arginine-based formulation concomitantly impacted the tertiary structure and the thermal stability of a mAb (mAb1). The secondary structure was not disrupted by the formulation change. The destabilization of the tertiary structure did not affect the long-term stability or the bioactivity of mAb1. This indicates that loss of conformational stability was likely compensated by improvements in the colloidal stability of mAb1 in the arginine-based formulation. The formulation-induced changes in HOS were reversible as proven by measurements after dilution in a common buffer (phosphate-buffered saline). For aggregation driven by assembly of aggregates (colloidally limited), small changes in conformational structure and stability as measured by HOS methods may not necessarily be predictive of long-term stability. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:1539–1542, 2015
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INTRODUCTION

Protein functionality is dependent on folding into the appropriate higher order structure (HOS). One of the current hypotheses regarding protein folding, the folding funnel, postulates that native state is associated with a minimum free energy and corresponds to a well-defined tertiary structure, under a specific solution condition.[1] However, from a thermodynamic perspective, proteins are only marginally stable and in a solution, they may rapidly interchange between an ensemble of close tertiary conformations.[2,3] During formulation development of protein therapeutics, conformational and colloidal stability are two of the important protein attributes that one tries to optimize. Ideally, the formulation should result in a drug with acceptable stability and, at the same time, meet the patient and healthcare provider needs for each development phase.[4–6]

In this note, we describe the outcome of a formulation change on multiple stability and conformational aspects of mAb1. mAb1 was originally developed as a lyophilized formulation (formulation A) with a protein target concentration of 50 mg/mL. Initial clinical data indicated that a higher protein concentration may be needed for future studies and subsequently, a liquid formulation (formulation B), with a protein target concentration of 150 mg/mL was developed. We show how FTIR, near-UV circular dichroism (NUV-CD), and differential scanning calorimetry (DSC) data were used to assess and understand the HOS of mAb1, following a formulation change. The three techniques were selected based on their ability to probe the secondary and tertiary protein structure changes as well as alterations of the intrinsic molecular thermal stability. Although the list of potential HOS tools is quite extensive, this subset of assays has historically proven to be sufficient for the usual structural investigations of mAbs.

MATERIALS AND METHODS

Protein Solutions

mAb1 materials were provided by the MedImmune Formulation Sciences Department. mAb1 liquid formulations, formulation A [25 mM histidine/histidine HCl, 225 mM trehalose dihydrate, 0.05% (w/v) polysorbate 80, pH6.0], and formulation B [25 mM histidine/histidine HCl, 150 mM arginine HCl, 0.07% (w/v) polysorbate 80, pH 6.0] were used without further manipulations. Vialed mAb1 lyophilized material (formulation A, lyo) was reconstituted following the recommended procedure.

FTIR Measurements

FTIR measurements were performed using a PerkinElmer Spectrum 100 spectrometer (Waltham, Massachusetts) in transmission mode. Samples were run at their original concentrations. At least 128 spectra from 3000 to 1000 cm^{-1} at 4 cm^{-1} resolution were recorded and averaged for the final result. The amide I band between 1700 and 1600 cm^{-1} was analyzed by the second derivative method.[7]

NUV-CD Measurements

Near-UV circular dichroism measurements were performed on a Jasco-815 instrument (Easton, Maryland). mAb1 solutions with a target optical density of 1.25 at 280 nm were prepared and run in duplicate in a 1-cm pathlength cuvette (1-Q10, Starna, Atascadero, CA). Spectra were collected at standard

Abbreviations used: HOS, higher order structure; NUV-CD, near-UV circular dichroism; PBS, phosphate-buffered saline.

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Table 1. Selected Stability Data for Formulations A lyo, Formulation A liquid, and Formulation B liquid

Formulation	Results of 1 Year Stability at 5°C			
	Purity Loss at 5°C (%/year)	Binding Assay Range (%)	Visual Inspection for Particles	Subvisible Particles by Flow Microscopy at 1 year (#/mL >10 μm)
50 mg/mL Formulation A lyo[a]	0.0[a]	98–104[a]	Practically free from visible particles[a]	38
50 mg/mL Formulation A liquid	0.6	100–106[b]	Practically free from visible particles	11
150 mg/mL Formulation B liquid	0.6	98–109	Practically free from visible particles	40

[a]Analyzed after reconstitution of lyophilized cake.
[b]Based on 6-month data.

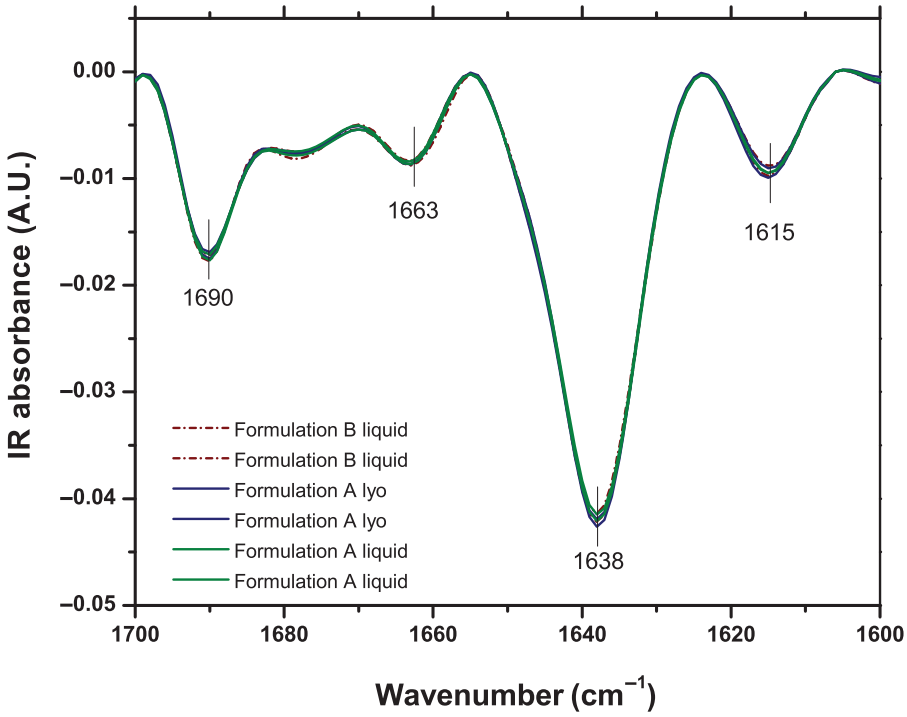

Figure 1. Second-derivative analysis of the FTIR amide I band of mAb1 in formulation A lyo (reconstituted), formulation A liquid, and formulation B liquid.

sensitivity range (100 mdeg) with 0.5 nm data pitch, 10 nm/min scanning rate, and 8 s integration time in the range from 250 to 350 nm. Two replicates were averaged for each sample to assess measurement variability.

DSC Measurements

Differential scanning calorimetry measurements were performed on a MicroCal VP-Capillary system (Northampton, Massachusetts). mAb1 solutions with a target protein concentration of 0.80 mg/mL were prepared and run in duplicate. Data were collected in the 20°C–95°C range using a temperature ramp-up of 60 °C/h. Two replicates were averaged for each reported thermogram to assess and control measurement variability.

Stability Studies

Four methods were selected for the evaluation of the long-term stability of each of the three formulations. Purity assessment was carried out by a high-performance size-exclusion chromatography method using a Tosoh Biosciences (Montgomeryville, Pennsylvania) G3000SWXL column. Purity is reported as the percent monomer peak. The purity loss rate is calculated from the slope of the purity versus elapsed time plot and is reported as percentage per year. Binding to the molecular target was evaluated by the use of a molecule specific surface plasmon resonance binding assay (Biacore C; GE Healthcare Bio-Sciences, Pittsburgh, Pennsylvania). Results are reported as percent relative bioactivity of the test sample relative to a reference sample. Subvisible particle formation was monitored

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by flow microscopy (MicroFlow Imaging, Brightwell Technologies, Ottawa), and visible particle formation was evaluated using procedures described in European Pharmacopeia 2.9.20, *particulate contamination: visible particles*.

RESULTS AND DISCUSSION

A summary of selected key stability data for reconstituted lyo product (formulation A), liquid product (formulation A), and liquid product (formulation B) placed in storage at 5°C for 1 year is reported in Table 1. The data show that all three formulations have demonstrated a stability profile that meets preestablished target shelf-life criteria (as measured by rate of purity loss, bioactivity, visual appearance and levels of subvisible particles). The lyophilized formulation was most stable and did not show any degradation after 1 year at 5°C. The rate of purity loss of both liquid products (formulations A and B) were low and comparable.

For HOS characterization of the three formulations, FTIR, NUV-CD, and DSC were used to assess changes in the secondary and tertiary structure and thermal stability, respectively.

The FTIR spectra display that all three samples had similar secondary structures (Fig. 1). A major band at 1638 cm^{-1} (intramolecular β-sheet) and bands at 1690, 1663, and 1615 cm^{-1}, characteristic of native or near-native IgG molecules, were observed for all three samples.

Near-UV circular dichroism spectra (Fig. 2, top panel) indicated that both formulation A lyo (postreconstitution) and liquid were comparable, whereas the spectrum of mAb1 in formulation B had a reduced molar ellipticity, suggestive of a loss of tertiary structure. The comparability was evaluated based on a normalized root mean square deviation based global comparison of the NUV-CD spectra coupled with statistical analysis of the point residuals.

Differential scanning calorimetry results (Fig. 3, top panel) showed that melting transition temperatures of mAb1 domains were significantly impacted by the change in formulation, similar to what was observed using NUV-CD. For both formulation A lyo and liquid, the melting transitions identified in the thermogram were identical ($T_m1 = 65.5 \pm 0.2$°C, $T_m2 = 71.7 \pm 0.2$°C, and $T_m3 = 84.3 \pm 0.2$°C), demonstrating that the lyophilization and reconstitution process in itself did not affect the tertiary structure. However, for formulation B liquid, all measured melting transitions were lower ($T_m1 = 60.8$°C, $T_m2 = 70.1$°C, and $T_m3 = 81.1$°C), consistent with a lower conformational stability of mAb1 in formulation B.

These results indicated that the formulation change did not have a measurable effect on the secondary structure of the mAb1 as measured by FTIR but it resulted in sizeable changes in the tertiary structure and overall conformational stability of the molecule as indicated by NUV-CD and DSC data. Although we observe a conformational destabilization for liquid formulation B (histidine–arginine), the long-term stability and activity was not impacted (Table 1). There are few past studies that report the use of arginine to improve solubility and refolding, reduce viscosity, and suppress the aggregation of proteins in a solution.[8,9] Although the mechanisms behind the observed arginine effects are not completely elucidated, its preferential binding to the protein was shown to play an important role.[10]

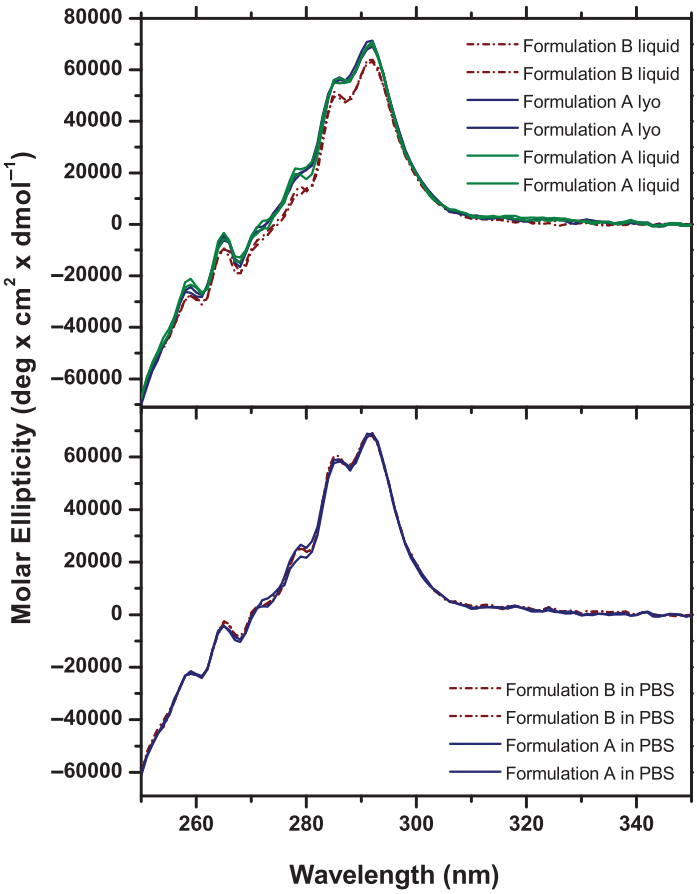

Figure 2. Overlays of the NUV-CD spectra of mAb1 in formulation A lyo (reconstituted), formulation A liquid, and formulation B liquid (upper panel). Overlays of the NUV-CD spectra of mAb1 in formulation A lyo (reconstituted) and formulation B liquid upon dilution into PBS (lower panel).

We have succinctly demonstrated how formulation composition may impact the tertiary structure of protein therapeutics without noticeable effects on the long-term stability or ability to bind to their intended target. However, we are still compelled to understand whether the formulation change has irreversible effects on mAb1 HOS. A possible resolution for this issue would be to test lots of mAb1 produced before and after the formulation change under common buffer conditions. Considering the clinical route of administration for mAb1, phosphate-buffered saline (PBS) was identified as a relevant option. mAb1 material sourced from both conditions (formulation A lyo and formulation B liquid) was diluted into PBS to the target assay concentration as a mimic for dilution in human plasma, and, subsequently, NUV-CD and DSC data were collected.

Near-UV circular dichroism data, shown in Figure 2 (lower panel), demonstrated that materials from the two processes assume similar tertiary structure in the common solution condition. The thermograms for formulation A lyo and formulation B liquid materials diluted in PBS are shown in Figure 3 (lower panel). For both mAb1 samples, melting transitions were very similar, with melting temperatures identified at 69.4 ± 0.2°C and 84.3 ± 0.2°C. To further characterize the conformation of the PBS-diluted samples, their binding to the intended target was tested as well and determined to be comparable

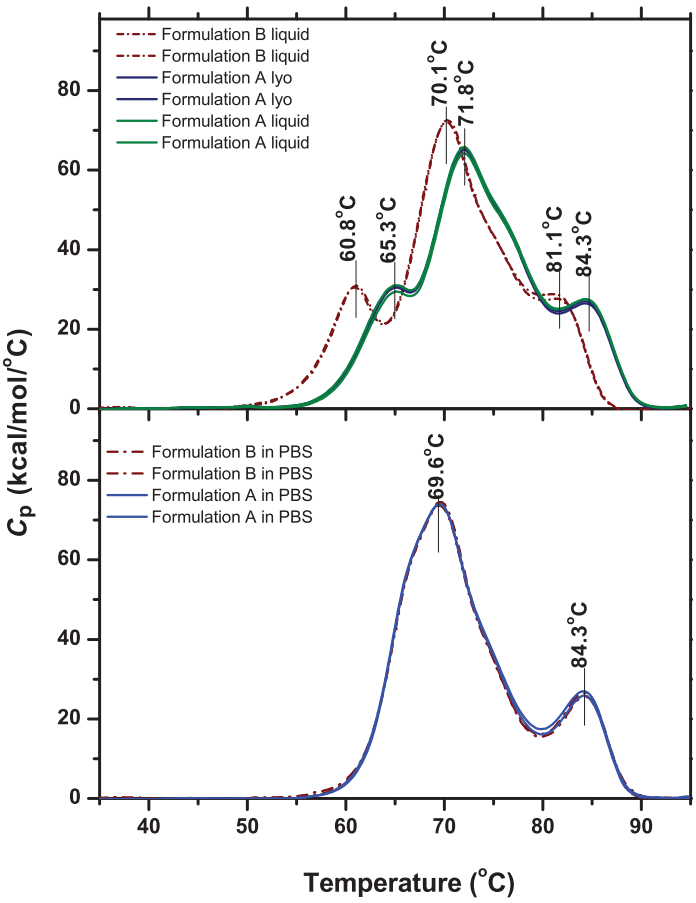

Figure 3. Overlays of mAb1 thermograms in formulation A lyo (reconstituted), formulation A liquid, and formulation B liquid (upper panel). Overlays of mAb1 thermograms in formulation A lyo (reconstituted) and formulation B liquid upon dilution into PBS (lower panel).

(97% for PBS-diluted formulation A and 100% for PBS-diluted formulation B, respectively). Therefore, the tertiary structure consistency for the two lots of mAb1 is demonstrated, and the reversibility and convergence of tertiary structure of formulations A and B diluted into PBS are established.

We propose that a similar strategy can be used for the comparability assessment of protein therapeutics, which undergo a formulation change. A variation of the experiment we presented would be to use the new formulation as the common solution condition for all samples to document the continuity of three-dimensional structure during product development. Regardless of what the common formulation is, the main requirement of insuring similar environment for the protein remains valid.

CONCLUSIONS

Different formulations may result in similar long-term stability profiles and acceptable bioactivity. However, the HOS of protein therapeutics can be impacted by the formulation conditions. In this case, the loss in tertiary structure and a decrease in conformational stability because of the formulation change were not predictive of long-term stability of mAb1, most likely due to the colloidal stabilization imparted by arginine. Although HOS differed for various solution conditions, the effects were reversible and the measured HOS converged when both formulations were assessed in a common solution condition. The use of a common condition (such as the new formulation) may be a good approach for assessment of reversibility and HOS comparability after a formulation change. For aggregation pathways driven by assembly of aggregates (colloidally limited), small changes in conformational structure and stability as measured by HOS methods may not necessarily be predictive of long-term stability. However, the HOS methods provided additional insights with regard to the protein characterization.

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