The Evaluation of Pressure-Assisted Enzymatic Digestion for the Optimal Digestion of Monoclonal Antibodies

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Novel Aspect: Reducing the time required for the characterization of therapeutic mAbs by bottom-up approaches.

Introduction

The characterization of monoclonal antibodies (mAbs) using peptide mapping is achieved through a combination of top-down and bottom-up approaches. For bottom-up studies, the mAb is typically digested with either trypsin or Lys-C, and the resulting peptides analyzed by HPLC-MS/(MS). In-solution digestion is the most common approach to achieve this goal, as sequence coverage and the assessment of low-level post-translational modifications is often required. Although effective, the in-solution digestion protocol is time consuming and overnight digestion is usually required. Pressure-assisted enzymatic digestion (PAED) is an emerging technology used to achieve comparable, or in some cases better, digestion efficiency in significantly less time. In this study, the effectiveness of PAED is evaluated at different conditions using an anti-Respiratory Syncytial Virus (anti-RSV) mAb.

Methods

The PAED was performed using a Pressure Biosciences NEP2320 Barocycler under two different conditions. In the first condition (native), the samples were mixed with enzyme, while in the second condition, the samples were first reduced and alkylated (R&A) on the bench using standard protocols, then mixed with enzyme. Replicate samples were digested in the barocycler for various intervals (2 hr max) using defined sequences of alternating high and low pressure. Replicate 18 hr in-solution digestion was also performed under both native and R&A conditions (with or without denaturation) as controls. All digests were analyzed using a Waters Acquity UPLC coupled with a Waters Premier Qq/TOF in DDA mode. Sequence coverage was determined by MASCOT search against an in-house database.

Preliminary Data

The preliminary results indicate the superiority of the PAED approach for the rapid enzymatic digestion of proteins. Sequence coverage of greater than 90% was achieved from both the native and the R&A samples within 60 min in the barocycler. The results obtained using the 60 min PAED approach were comparable to those obtained from the control sample that was denatured, reduced and alkylated followed by overnight digestion. When the model mAb sample was only reduced and alkylated, without the denaturing step, no peptides were identified under the same database search criteria. This indicates the effectiveness of the PAED approach in reducing the total cycle time required for the characterization of mAbs. Moreover, the reproducibility of the PAED approach was confirmed by replicate analysis. In this study, the effect of the different experimental conditions on various success criteria, including but not limited to, sequence coverage, number of missed cleavages and the number of unmatched peptides, was investigated and will be presented.