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

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The characterization of monoclonal antibodies (mAbs) using peptide mapping is achieved through a combination of topdown 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 posttranslational 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. The preliminary results indicate the effectiveness of the PAED approach for the rapid enzymatic digestion of mAb. When the reduction and alkylation of the mAb was performed in PBS, results obtained using the 60 min PAED approach were comparable to those obtained from the overnight-bench top digestion. In addition, the PAED approach is very suitable for native digestions, which is important for disulfide mapping. When the sample was R&A in a denaturing buffer containing GuHCl, 80% sequence coverage was obtained within 20 min of pressure cycling. Performing the reduction and alkylation in a denaturing buffer also decrease the number of under-cleaved peptides. The PAED approach is very effective in reducing the total time required to digest proteins and will expedite the complete characterization of mAbs.

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