Purified Protease Type XIII for Enhanced Sequence Coverage in Hydrogen Deuterium Exchange Mass Spectrometry

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INTRODUCTION

Hydrogen deuterium exchange coupled to mass spectrometry (HDX-MS) has emerged as a powerful tool in protein structural and dynamics characterization. A critical and time-consuming step in HDX-MS is to efficiently digest the target protein and achieve sufficient sequence coverage. While pepsin is widely used as the primary protease in HDX experiments, it is often challenging to obtain complete sequence coverage using pepsin alone. Protease type XIII from Aspergillus saitoi has been shown to be a useful reagent complementary to pepsin. Here we show that the commercial available protease XIII suffers from low purity and proteolytic activity and seek to improve the properties by chromatographic purification of the enzyme to homogeneity.

METHODS

- The crude protease XIII from the commercial source was subjected to a three-step chromatographic purification process.
- The purity of the prepared enzyme was assessed on SDS-PAGE and size exclusion chromatography (SEC).
- The proteolytic activity was assessed by the hemoglobin TCA assay.
- Digestion of protein substrates was carried out at 4 degree for 8 min and injected to LC-MS for sequence coverage determination.
- The LC components are maintained at 0 degree during the analysis.

RESULTS

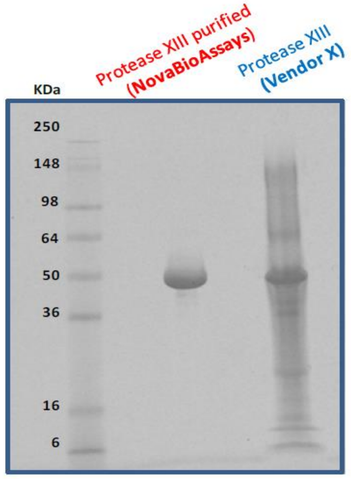

Figure 1. 1D SDS-PAGE gel image for crude and purified protease XIII.

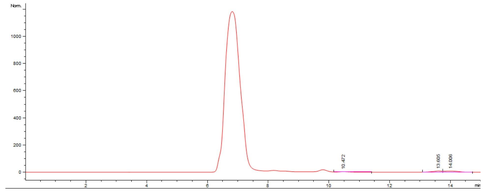

Figure 2. SEC chromatogram shows that the purified protease XIII are free of aggregation**.**

The proteolytic activity of the purified protease XIII was found to be ~1.5 folds higher than porcine measured using the hemoglobin TCA protocol.

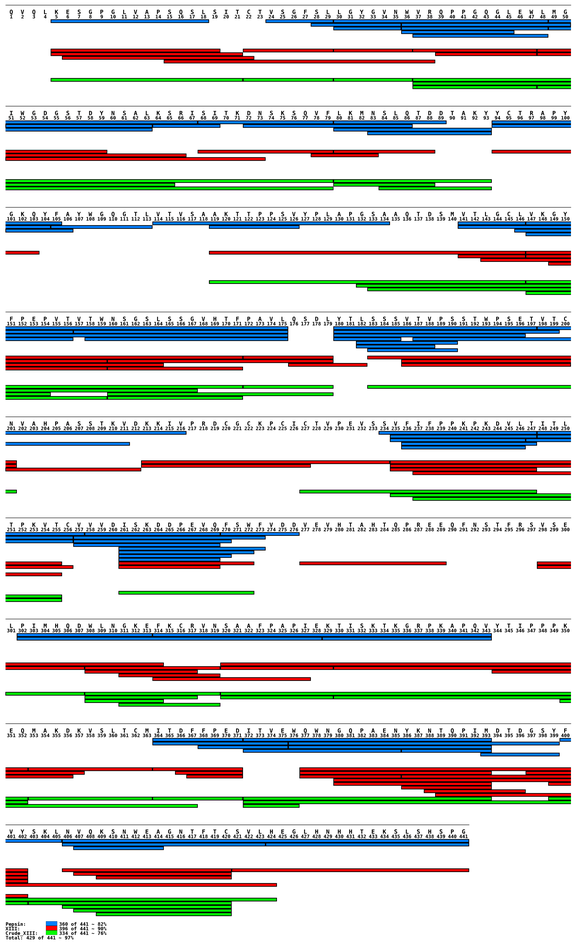

Figure 3. Sequence coverage of the heavy chain of a commercial mAb cleaved by pepsin (blue underlined), purified protease XIII (red underlined), and crude protease XIII (green underlined).

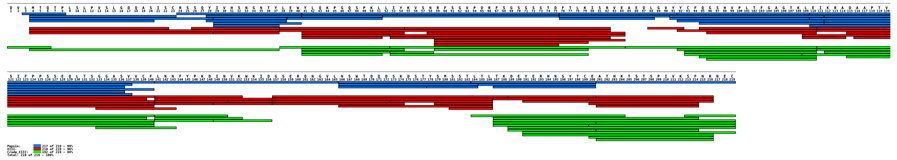

Figure 4. Sequence coverage of the light chain of a commercial mAb cleaved by pepsin (blue underlined), purified protease XIII (red underlined), and crude protease XIII (green underlined).

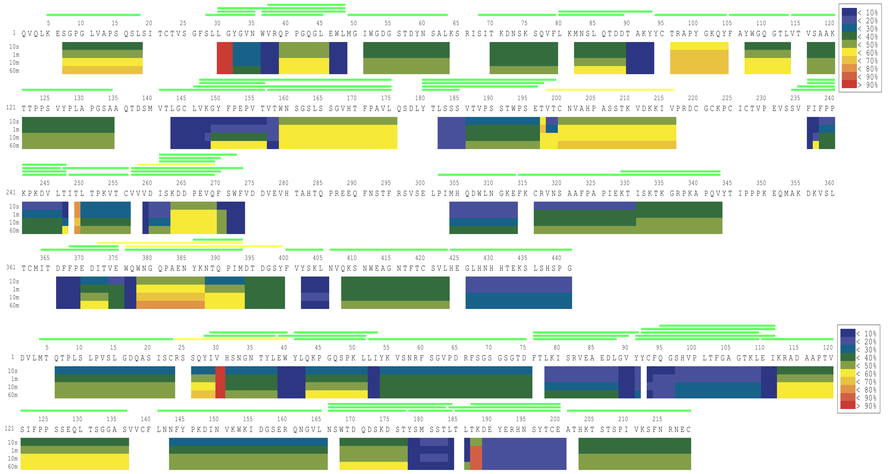

Figure 5. H/D exchange of a commercial mAb at pH7.2

CONCLUSIONS

- A three-step chromatographic separation significantly increases the purity of protease type XIII.
- Purified protease type XIII are free of aggregation and the proteolytic activity of the enzyme was found to be around 1.5 folds higher than porcine pepsin.
- Purified protease type XIII is superior to crude protease type XIII and pepsin based on our results for a mAb and several other proteins, by producing more fragments and better sequence coverage.