TOSOH

APPLICATION NOTE

PURIFICATION OF ACTIVE PEPTIDASE A USING A SEMI-PREPARATIVE TSKgel® G3000SW

INTRODUCTION

Enzymes are a highly versatile group of proteins which find applications ranging from medicine through waste degradation to food technology. Their safe application requires thorough characterization, which needs sufficient amounts of purified enzyme. The purification process is both an important, yet complicated step in enzyme production, especially for unmodified proteins lacking any means of selective binding. On the other hand, the introduction of such domains (e.g.a His-tag) may influence the enzymes characteristics and is therefore often not desired. Such constraints leave a very limited choice of purification methods. In this study, recombinant aminopeptidase A (PepA) expressed in E.coli was purified. This enzyme forms dodecamers which suggested size exclusion chromatography (SEC) as a suitable separation technique. The high loading capacity of the TSKgel G3000SW columns series is demonstrated, as well its superior separation performance compared to a competitor column. Furthermore, a larger column of the G3000SW series was used for the semi-preparative amount of 14 mg of protein extract, showing that the G3000SW column is suitable for upscaling purposes. (For a detailed report see *Stressler et al., Protein Expression and Purification 131 (2017) 7-15.*

PURIFICATION OF PepA USING A TSKgel G3000SW (TOP) AND A COMPETITOR COLUMN (BOTTOM)

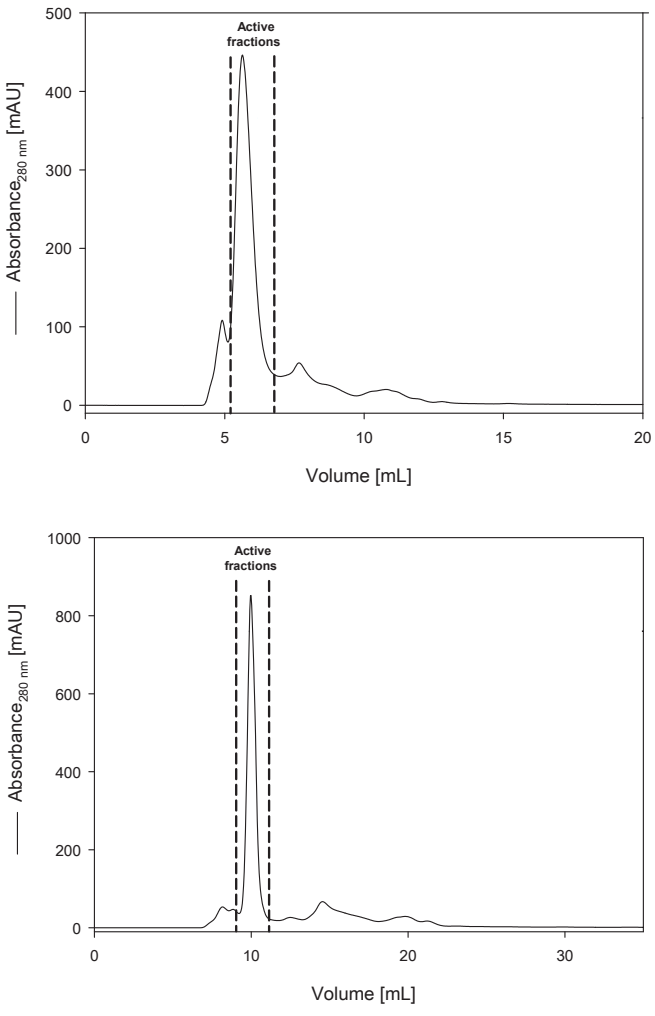

Figure 1

RESULTS AND DISCUSSION

After ammonium sulfate precipitation, the PepA showed a purity of approximately 95% (data not shown). For the subsequent purification SEC seemed promising due to the native molecular mass of approximately 480 kDa (homo dodecamer with ca. 40 kDa per subunit). Figure 1 shows the purification of PepA using the analytical TSKgel G3000SW column compared to a competitor column.

EXPERIMENTAL CONDITIONS

Columns:	TSKgel G3000SW (10 µm; 7.5 mm ID x 30 cm L; P/N 0005789) Competitor column (8.6 µm; 10 mm ID x 30 cm L) TSKgel G3000SW (13 µm; 21.5 mm ID x 60 cm L; P/N 0005147) and SW Guard (13 µm; 21.5 mm ID x 75 mm L; P/N 0005758)
Mobile phase:	50 mM Na/K-phosphate buffer pH 6.0
Flow rate:	Small scale: 0.5 mL/min Semi preparative scale: 6 mL/min
Detection:	UV @ 280 nm
Sample:	Recombinant aminopeptidase A (PepA) ammonium precipitate
Injected amounts:	Small scale: 0.3 mg/mL column resin (4.1 mg (TSKgel G3000SW) and 7.1 mg (competitor column) Semi preparative scale: 14 mg
System:	ÄKTA fast protein liquid chromatography
Detection:	UV @ 280 nm
Activity test:	H-Asp-pNA activity assay (data not shown)

As seen in Figure 1, a defined peak containing PepA was observed for both columns. To evaluate the particular purifications, a subsequent SDS-PAGE analysis was performed (see Figure 2).

SDS-PAGE ANALYSIS OF THE PepA ACTIVE FRACTIONS AFTER PURIFICATION USING AN ANALYTICAL TSKgel G3000SW (A) AND A COMPETITOR COLUMN (B)

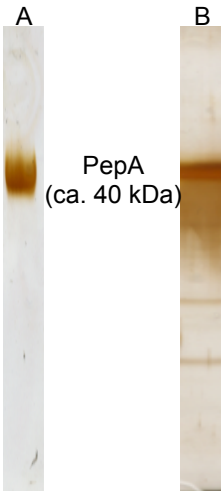

Figure 2

Although the PepA peak was sharper using the competitor column, the purity (see Figure 2) was higher for the TSKgel G3000SW column. Only a single band was observed when using the TSKgel G3000SW column. In contrast, besides PepA, additional bands were observed on SDS-PAGE after purification using the competitor column.

Due to the higher purity obtained with the analytical TSKgel G3000SW column, up-scaling of the purification process was performed with a preparative TSKgel G3000SW column.

PURIFICATION OF PepA USING A PREPARATIVE TSKgel G3000SW COLUMN

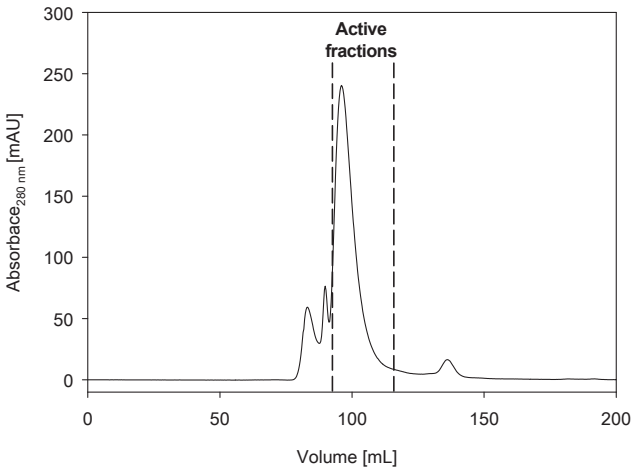

Figure 3

As seen in Figure 3, the purification chromatogram of the preparative TSKgel G3000SW was similar to that obtained using the analytical TSKgel G3000SW column (Figure 1). In addition, the subsequently performed SDS-PAGE was identical to Figure 2 (lane A). This indicates that up-scaling from the analytical to the preparative TSKgel G3000SW was possible without losing purity of the PepA.

CONCLUSION

This work illustrates that the series of TSKgel G3000SW columns was useful for the purification of an enzyme with a high molecular mass of approximately 480 kDa. The purity was higher than using a comparable competitor column. In addition, up-scaling of the purification process was possible without losing purity.