Using the Agilent 2100 bioanalyzer for analysis of His-tag removal from recombinant proteins

Application

Annette Schäfer
Frank Schäfer

Abstract

The TAGZyme system allows efficient and convenient exoproteolytic cleavage of N-terminal tags, for example, the His-tag, from recombinant proteins. This Application Note describes how the efficiency and kinetics of the cleavage process was analyzed in a fast and precise way using the Protein 200 LabChip kit and the Agilent 2100 bioanalyzer. Data on the processing of 6xHis-tagged GFP (Green Fluorescent Protein) and IL-1β (Interleukin-1 beta) proteins is presented here as part of a comprehensive analysis of the TAGZyme system[1].

Agilent Technologies

Introduction

The precise enzymatic removal of tags from recombinant proteins is an important tool for generating proteins with natural amino acid composition, i.e. sequence[2]. While short affinity tags such as the 6xHis tag in combination with Ni-NTA (nickel nitrilo triacetic acid), IMAC (immobilized-metal affinity chromatography) technology greatly simplify and speed up protein purification[3,4] and do not normally affect protein structure or function, their removal may be desired for some applications, for example the production of pharmaceuticals or protein crystallography/X-ray structure analysis.

While most approaches to remove affinity tags rely on endoproteolytic cleavage of proteins at a given recognition site, i.e. amino acid sequence[2], the TAGZyme system enables efficient and precise exoproteolytic cleavage of a protein and thereby shows no unspecific or unwanted internal cleavage[1]. The system uses dipeptidyaminopeptidase (DAPase) to progressively cleave off dipeptides from the N-terminus until a specific stop point is reached. Once the cleavage is complete, the His-tagged TAGZyme enzymes can be removed by subtractive IMAC using Ni-NTA matrices[5].

The Agilent 2100 bioanalyzer was used for fast and convenient analyses when exact data on the kinetics of cleavage reactions was required.

Results and Discussion

The kinetics of TAGZyme (QIAGEN GmbH, Hilden, Germany) cleavage of proteins with His-tags of different lengths and from different proteins were investigated using Agilent's lab-on-a-chip microfluidic system, the Agilent 2100 bioanalyzer (Agilent Technologies Deutschland GmbH, Waldbronn, Germany). Two 6xHis GFP variants (A) and (B) and 6xHis human IL-1β (1) were processed and analyzed on chips. Figure 1 shows the gel-like image and electropherograms demonstrating the cleavage kinetics delivered by the 2100 bioanalyzer system and the Protein 200 assay*. GFP (A) contains a long tag of 24 amino acids that was cleaved after 30 minutes. GFP (B) contains a short tag of 10 amino acids that cleaved after 15 minutes (figure 1A). Complete cleavage was confirmed by N-terminal sequencing[1].

For both GFP variants, DAPase revealed similar cleavage rates of 1.0-1.5 amino acids per minute, respectively (table 1) indicating that cleavage rate is independent of tag size. This cleavage rate corresponds to a substrate turnover of 0.63 and 0.47 µmol dipeptide per minute per unit DAPase. For IL-1β, cleavage was found to be complete within 30 minutes (figure 1A) with a cleavage rate of 2.0 amino acids per minute, equivalent to a substrate turnover of 0.44 µmol/min/U DAPase (table 1). Turnover rates of DAPase for different protein substrates and tag sizes were found to be very similar. This data is in accordance with the turnover of 1 µmol/min/U under optimized assay conditions[6] and show the high processivity of N-terminal dipeptide cleavage by DAPase. Target protein cleavage is typically complete within 30 minutes under standard conditions[6].

Protein	Size (kDA)	Tag size (amino acids)	Processed protein (µmol)	Substrate cleavable dipeptides (µmol)	Cleavage complete in	Cleavage per turnover amino acid	Substrate time (µmol/min × unit)
GFP (A)	30	24	0.033	0.792	25 min	1.0	0.63
GFP (B)	28.5	10	0.035	0.350	15 min	1.5	0.47
IL-1β	21	14	0.047	0.658	30 min	2.0	0.44

Table 1
Kinetic data from DAPase cleavage. Calculations are based on the following parameters: mean molecular weight per amino acid: 110 g/mol; 1 mg of protein has been processed using 50 mU DAPase, respectively.

* The improved Protein 200 Plus LabChip kit replaced the Protein 200 LabChip kit and shows the same performance.

Conclusion

The Agilent 2100 bioanalyzer is a powerful analysis tool enabling characterization of both purity and size of several proteins in parallel within less than 45 minutes. The Protein 200 assay has a wide separation range from 14 to 200 kDa. The system proved to be very suitable for the separation and analysis of protein size differences of as little as 10 amino acids. The use of the electropherogram display allowed exact determination of the cleavage kinetics even if the processing of a very small affinity tag was analyzed. Availability of kits with a narrower separation range may facilitate such studies even more.

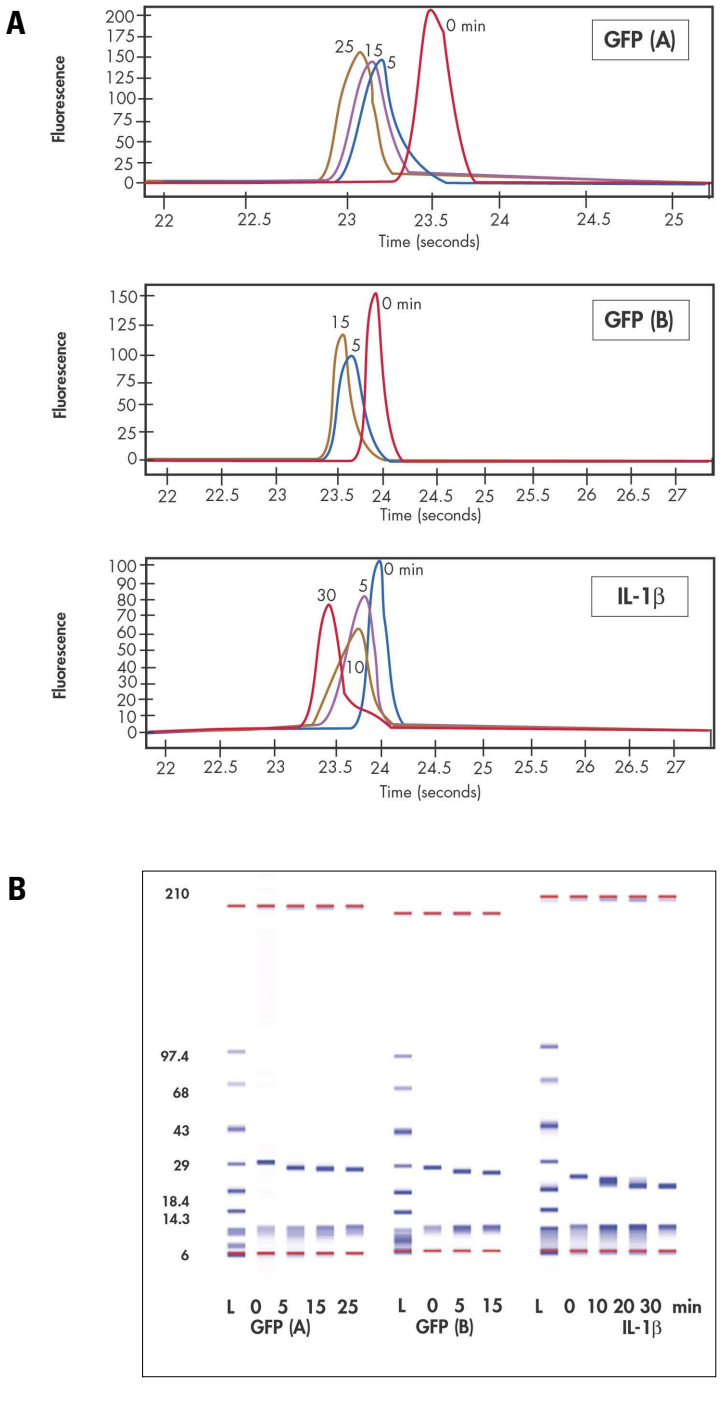

Figure 1
Time-resolved representation of DAPase cleavage. Processing of GFP variants A and B and IL-1β using the fluorescence-based detection with the Agilent 2100 bioanalyzer.
A) Electropherogram to determine time needed for complete digestion.
B) Gel-like image of analyzed digestions. The red bars represent the high and low molecular weight markers for internal normalization of the lanes. L: Molecular weight ladder (kDa). Cleavage reactions containing 1 mg 6xHis-tagged protein and 50 mU DAPase were set up under standard conditions (6). Samples (1 µg) were taken at the indicated time points and analyzed with the Protein 200 assay according to the manufacturer's recommendations. The Agilent 2100 bioanalyzer data analysis software was used to zoom into the electropherogram peaks representing the protein bands. Earlier detection of the peaks corresponds to a progression of N-terminal cleavage.

References

1.
Schäfer, F., Schäfer, A., and Steinert, K. **(2002)** "A highly specific system for efficient enzymatic removal of tags from recombinant proteins." *J Biomolec Techniques 13, 158-171.*

2.
Nilsson, J., Ståhl, S., Lundberg, J., Uhlén, M., and Nygren, P.-Å. **(1997)**. "Affinity fusion strategies for detection, purification, and immobilization of recombinant proteins." *Protein Express Purif 11, 1-16.*

3.
Hochuli, E., Döbeli, H., and Schacher, A. **(1987)**. "New metal chelate absorbent selective for proteins and peptides containing neighbouring histidine residues." *J Chromatog 411, 177-184.*

4.
Janknecht, R., de Martynoff, G., Lou, J., Hipskind, R. A., Nordheim, A., and Stunnenberg, H. G. **(1991)**. "Rapid and efficient purification of native histidine-tagged protein expressed by recombinant vaccinia virus." *Proc Natl Acad Sci USA 88, 8972-8976.*

5.
"TAGZyme System - efficient and highly specific removal of His tags by exoproteolytic peptidases." **(2001)**. QIAGEN News 4, 1-8: www.qiagen.com/literature/qiagennews/0401/1018052_QNews42001_P1_6_8.pdf

6.
"TAGZyme handbook for exoproteolytic cleavage of N-terminal His tags" (**06/2001**). QIAGEN, Hilden, Germany.

Annette Schäfer is Laboratory Technician and Frank Schäfer is Senior Scientist in the Department Protein Expression/Proteomics at QIAGEN GmbH, Hilden, Germany.

www.agilent.com/chem/labonachip

Published October 1, 2002
Publication Number 5988-8144EN

Agilent Technologies