Using the Agilent 2100 bioanalyzer for quality control of protein samples prior to MS-analysis

Application

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Introduction

Whereas in the past mass spectrometers (MS) were confined to the realm of small molecules, the development of soft ionization methods such as matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI) revolutionized the analysis of large biomolecules[1, 2]. Today, the analysis of proteins up to 300 kDa using MS has become a routine method to gain molecular weight information with high accuracy and structural information. However, sample purity greatly influences the results of MS analysis. In MS service facilities, scientists have to rely on the information provided by the customers and the quality of the sample preparation they have done. Bad results are often the consequence of customers being too optimistic about their own samples. Especially concentration and purity are often overestimated. To avoid unproductive MS runs, a fast and simple precheck is valuable. In this Application Note we show that the Agilent 2100 bioanalyzer can be used for quality control of protein samples prior to MS analysis. A corresponding data set analyzed with LC/MS and with the Agilent 2100 bioanalyzer using the Protein 200 Plus LabChip® kit is shown.

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Experiments

All samples were adjusted to a concentration of 1 mg/mL by dilution with the appropriate buffer prior to the analysis (based on information given by the customer).

LC-MS analysis

The experimental setup comprised:

- Micro-HPLC-Pump (ABI 140C)
- Autosampler (PE200)
- HPLC column (Nucleosil C8 125 x 2 mm, 5 µ, 120 Å from Macherey & Nagel)
- UV detector (ABI 785A) set to 214 nm, in row with
- Single quadrupole mass spectrometer (API165 from Applied Biosystems) with a 1:10 flow splitter

The analysis was performed as follows:
10 µL sample was injected and the proteins were separated with a flow of 250 µL/min. A linear gradient was applied using eluent A (0.1 % TFA in water) and eluent B (0.1 % TFA in acetonitrile) from 5 % eluent B within 15 miutes to 95 % eluent B. The gradient was started with 20 % eluent B when analyzing samples with low salt or very hydrophobic proteins. For MS detection an ESI-spray source was used in positive ion mode with an ionization voltage of 5000 V and an orifice potential of 40 V. The analysis was carried out with a full scan between 600 and 1800 m/z (2.7 s/scan). For deconvolution the "Biotoolbox" software was used.

Agilent 2100 bioanalyzer and the Protein 200 Plus assay

Samples and chips were prepared according to the protocol provided with the Protein 200 Plus LabChip kit. The kit includes 25 chips, spin filters and all reagents needed for the experiments including the Agilent Protein 200 Plus ladder and the upper and lower marker premixed in the sample buffer. The chip-based separations were performed on the Agilent 2100 bioanalyzer using the dedicated Protein 200 Plus software assay. The software automatically determines the size of each protein in kDa, its relative concentration to the upper marker and the percent total of the protein. Latter gives direct information on the purity of a particular protein.

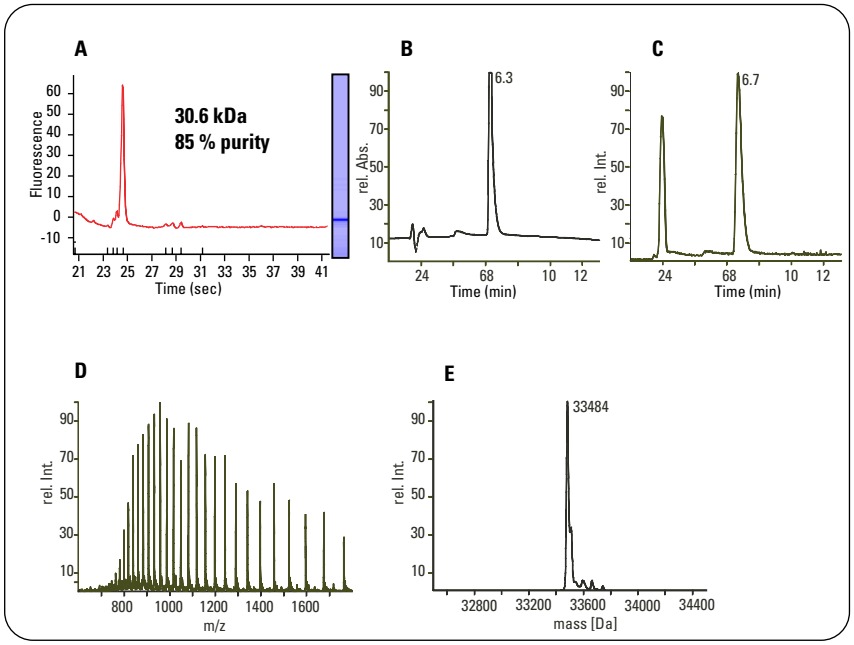

Figure 1
Results of the analysis of protein 9-15 with the Agilent 2100 bioanalyzer and the LC/MS method, expected molecular weight (MW) 33484 Da. A. Agilent 2100 bioanalyzer electropherogram B. UV-signal at 214 nm C. Total ion current (TIC) D. Raw mass spectrum E. Deconvoluted mass spectrum

	Expected MW [Da] (provided by customer)	MW by MS [Da]	MW by bioanalyzer [kDa]
Sample 2-11	26111	26237 (additional Glu)	24.5
Sample 3-12	26392	26405	24.5
Sample 9-15	33484	33484	30.6
Sample 16	~30000	~30000 is present	2 main peaks at 65.9/131.9, small peak at 30
Sample 21	~47880 + multiple Label (n x 180Da)	47889 + n x 180Da (n=0,1,2)	43.7 + multimers
Sample 22	~70000	71471	main peak at 60.9, smaller peak at 48.5
Sample 23	33484	33483	30.6

Table 1
Comparison between the expected molecular weight (MW), MW determined with MS-analysis and Agilent 2100 bioanalyzer.

Results and discussion

Several protein samples were analyzed both with the LC/MS method (optimized for robust online desalting and rough separation of impurities) as well as the Agilent 2100 bioanalyzer and the Protein 200 Plus assay. A summary of the results is shown in table 1.
The protein sample 9-15 for example did not cause any problem during MS analysis (figure 1). It was purified by the customer using a gel-column and was provided in a low salt buffer (50 mM Tris/HCl, 1 mM EDTA). A molecular weight of 33484 Da was expected. The UV-chromatogram showed a single peak at 6.3 minutes (figure 1B), which corresponds to the peak at 6.7 min in the TIC (figure 1C). After deconvolution of the correlated mass spectrum (figure 1D) the protein mass was found to be exactly as expected (figure 1E). Also, the 2100 bioanalyzer electropherogram (figure 1A) showed a main peak at 30.6 kDa with a purity of 85.0 %, which corresponds nicely to the MS results.
For protein sample 16 the results were already more difficult to interpret (figure 2). The protein was purified with a Ni-NTA-column and was provided in a buffer with relatively high ionic strength compared to the protein sample 9-15 (50 mM NaH_2PO_4, 250 mM imidazole, 300 mM NaCl). A molecular weight of approximately 30 kDa was expected. The UV chromatogram showed a main peak at 4.7 minutes and some minor peaks at 7.4 minutes and 11.2 minutes However, the TIC showed two prominent signals (figure 2C) – one at 5.1 minutes, which corresponds to the 4.7 minutes signal

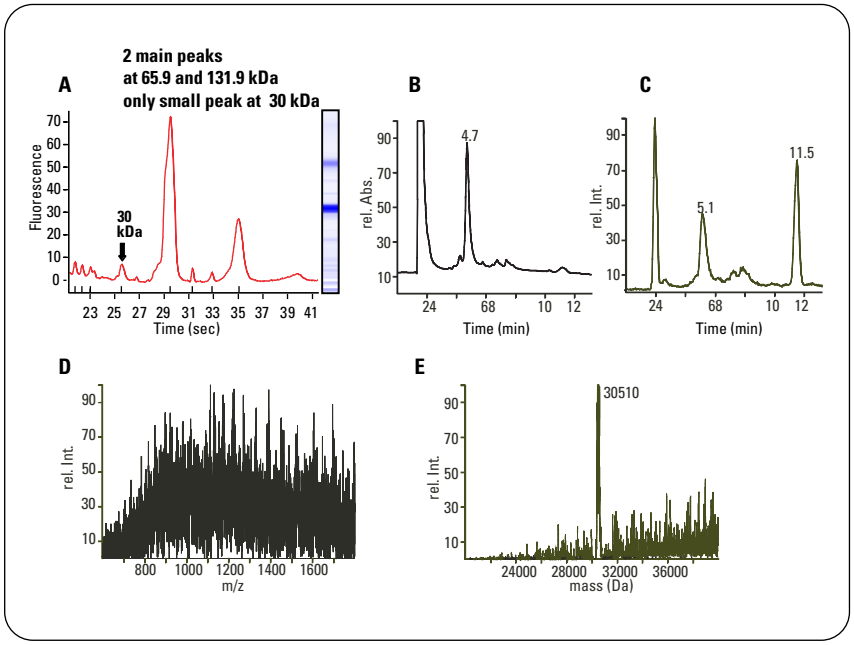

Figure 2
Results of the analysis of protein 16 with the Agilent 2100 bioanalyzer and the LC/MS method, expected molecular weight (MW) 30 kDa. A. Agilent 2100 bioanalyzer electropherogram B. UV-signal at 214 nm C. Total ion current D. Mass spectrum (5.1 min) E. Deconvoluted mass spectrum (5.1 min)

in the UV, and a second peak at 11.5 minutes, which seemed to be only a minor impurity in the UV but was obviously easy to ionize. Both TIC signals were analyzed, where the first mass spectrum turned out to be very noisy (figure 2D), which is usually an indication for inhomogenious samples and/or a high tendency for aggregation. As expected, the deconvolution yielded several mass peaks of which the most intense around 30 kDa could only be considered as a hint that the expected protein is present. The second mass spectrum showed a polymer pattern between 600 and 900 m/z (data not shown). However, the data from the analysis with the Agilent 2100 bioanalyzer already showed that only a small peak of the expected 30 kDa protein is visible in the electropherogram (figure 2A). Instead, the analysis revealed two main peaks at 65.9 kDa and 131.9 kDa.

Conclusion

The presented data showed that there is a good correlation between the results determined with the Agilent 2100 bioanalyzer and MS-analysis. Since the Agilent 2100 bioanalyzer provides a fast, standardized method for protein analysis with automated and detailed data analysis, it is an ideal tool for quality control prior to MS analysis. In this case, the LC/MS method lasted approximately 20 minutes and additional 10 minutes are needed for column equilibra-

tion and sample preparation.By comparison, the Agilent 2100 bioanalyzer can analyze 10 samples within 45 minutes including sample preparation. Depending on the kit, proteins from 5-200 kDa can be analyzed with a resolution of 10 %, or better, throughout the size range[3, 4]. However, sizing accuracy is strongly dependent on the protein characteristics, which is also the case for other methods such as traditional gel-electrophoreses (SDS-PAGE) or size exclusion chromatography. Sizing reproducibility is in the range of 0.5–5 %. In contrast, excellent mass accuracy of 0.01 % and very high sensitivity can be obtained by mass spectrometry if the protein sample fulfills the purity criteria needed for the analysis. Comparing the sizing results from the Agilent 2100 bioanalyzer with those obtained by MS, the absolute error of size determination with the Agilent 2100 bioanalyzer ranges from 6–14 % for the proteins analyzed in this study. With the help of the Agilent 2100 bioanalyzer "dirty" samples can be identified and only "clean" samples with the right concentration will be subjected to MS analysis. This pre-screening reduces costs and significantly saves time. Furthermore, the Agilent 2100 bioanalyzer can reveal additional information, such as concentration determination or the formation of multimers, which was not possible with the LC/MS method described.

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