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Application News

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MALDI-TOF Mass Spectrometry

Quantitative Analysis of Animal Hair Fibers Using a Benchtop MALDI-TOF Mass Spectrometer

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometers (MALDI-TOF MS) feature simple and rapid acquisition of molecular weight information from a wide range of macromolecular samples, including peptides, proteins and synthetic macromolecules. MALDI-TOF MS is widely used to determine the molecular weights of synthesized products and natural substances in R&D laboratories and on site in the quality control field.

One of the new uses of MALDI that has been advocated is a method for differentiation of animal hair by detecting species-specific peptides in animal hair [1]. This differentiation method is approved by the International Organization for Standardization (ISO) as a "method for proteomic analysis of cashmere and some other animal hair fibers" and is defined by an ISO standard [2]. This differentiation is considered to have the potential for application not just to fake cashmere but also to contamination of food etc., by inclusion of foreign matter, since analysis is possible even with just a single hair.

This article introduces an example of quantitative analysis of animal hair fibers using a MALDI-8020 benchtop MALDI-TOF MS.

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Research Method

Pretreatment of the samples was carried out in accordance with the method provided in the ISO standard. [2] The outline of the pretreatment procedure is as follows. Raw hair of cashmere and sheep wool was cut with scissors and crushed to a fine powder with a ball mill. Next, 4 % sodium dodecyl sulfate (SDS) containing 50 mM of dithiothreitol (DTT), and 0.5 ml of 0.1 M of a phosphate buffer solution (pH 7.8) were added to 10 to 50 mg of the crushed raw hair, and the solution was then heated at 95 °C for 15 min to 1 h. After heating, iodoacetamide was added to the extract solution to adjust the concentration to 100 mM. The solution was then reacted at room temperature for 15 min, after which the reaction was stopped by adding 10 µL of 25 mM DTT. The extract was separated and stained by SDS polyacrylamide gel electrophoresis (SDS-PAGE), and gel pieces containing the target protein were cut out. After removing the stain Coomassie Brilliant Blue (CBB) by using 50 mM ammonium bicarbonate/50 % acetonitrile, the sample was digested at 50 °C for 1 h by adding approximately 150 ng of trypsin.

Fig. 1 MALDI-8020 Benchtop MALDI-TOF MS

Digested samples were desalted using a ZipTip® µC18 (Merck Millipore). The desalted sample solution was spotted on the MALDI target plate, then, 0.5 µL of the matrix solution was overlaid on the sample, and mass spectrometry was conducted. The matrix was a solution in which CHCA (α-cyano-4-hydroxycinnamic acid) was dissolved in 50 % acetonitrile/0.05 % trifluoroacetic acid (TFA) so as to obtain a concentration of 5 mg/mL.

A MALDI-8020 benchtop MALDI-TOF MS (Fig. 1) was used for analysis and the measurements were done in the linear positive mode.

Results

Fig. 2 shows the mass spectra of trypsin-digested peptides from specimens of cashmere mixed with sheep wool at various ratios from 10 % to 90 %. The cashmere- and wool-specific peptide peaks were detected at *m/z* 2691 and *m/z* 2664, respectively. It can be understood that the intensity ratio of these peaks changes corresponding to the mixing ratio. It is possible to identify the type of animal hair and measure the mixing ratio by using these species-specific peaks. It may be noted that the main protein that makes up animal hair is keratin, and the peptides detected here are also derived from keratin.

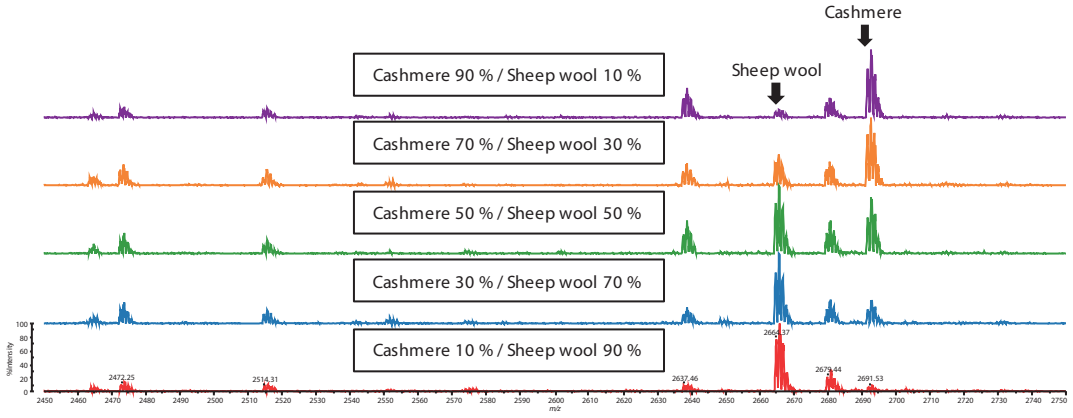

Fig. 2 Mass Spectra of Trypsin-Digested Peptides of Cashmere-Sheep Wool Mixtures

Table 1 Amino Acid Sequence of Animal Species-Specific Region in Keratin Type I [1]

Species	Keratin Type I Protein	Amino acid sequence	$[M+H]^+$ Monoisotopic mass *1
Cashmere	Keratin 33A [*Capra hircus*]	YSCQLNQVQSLIVNVESQLAEIR	2691.38
Yak	Keratin type I microfibrillar, 47.6 kDa-like [*Bos mutus*]	YSSQLAQVQGLIGNVESQLAEIR	2503.32
Sheep wool	Keratin 33B [*Ovis aries*]	YSCQLSQVQSLIVNVESQLAEIR	2664.37
Horse	Keratin 33A [*Equus caballus*]	YSSQLSQVQGLITNVESQLAEIR	2563.34
Dog	Keratin 33A [*Canis lupus familiaris*]	YSSQLNQVQCMITNVESQLAEIR	2711.31
Brown rat	Keratin 31 [*Rattus norvegicus*]	YSSQLSQVQCLITNVESQLGEIR	2652.33
Human	KRT34 protein [*Homo sapiens*]	YSSQLSQVQSLITNVESQLAEIR	2593.35

*1 For the cysteine residue, the monoisotopic mass is calculated as the carbamidomethylated (by iodoacetamide) form.

Table 1 shows the assignment results for the animal species-specific peaks.[1] These amino acid sequences originate from keratin type I proteins. In addition to the animal species measured in this experiment, it can be understood that these sequences are also specific for humans, brown rats and other species.

Next, the peak intensity ratios were obtained by the following equation using the intensities of the cashmere- and sheep wool-specific peaks.

Cashmere Peak (%) = PAc * 100/(PAc + PAw)

where, PAc : intensity of cashmere-specific peak
PAw : intensity of sheep wool-specific peak

Fig. 3 shows calibration curves in which the relationship of the obtained intensity ratios and mixing ratios is plotted. In order to confirm reproducibility, SDS-PAGE of the same samples was done in two series, and calibration curves were prepared for each (red line and blue line in Fig. 3). Satisfactory linearity was confirmed for both prepared calibration curves, and the coefficient of correlation was close to 1. These results showed that it is possible to measure the mixing ratio of animal hair fibers, beginning with cashmere and sheep wool, by using the MALDI-8020 benchtop MALDI-TOF MS.

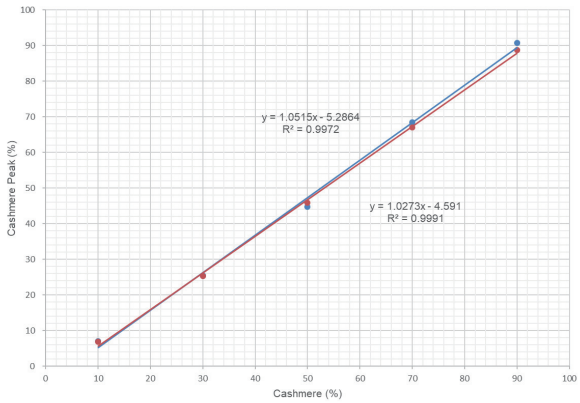

Fig. 3 Calibration Curves of Cashmere/Sheep Wool Mixtures

Conclusion

This experiment demonstrated that quantitative analysis of animal hair fibers is possible by using the MALDI-8020 benchtop MALDI-TOF MS. It has been shown that quantification of the mixing ratio of animal hairs in commercially-available fiber products made from cashmere, sheep wool and yak as the raw materials is possible by MALDI using the technique described in the reference,[1] and similar quantitative analysis using the MALDI-8020 is also considered possible.

In the future, further development of the MALDI-8020, which offers a compact benchtop footprint and the performance necessary for identification of animal hairs, is expected as a product that enables simple and quick evaluation of diverse types of animal hair fiber products.

<Acknowledgments>
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<References>
(1) Shinichi Ohashi *et al.*: Quantitative Analysis of Cashmere and Other Animal Hair Fibers in Textiles Using MALDI-TOF Mass Spectrometry, SEN'I GAKKAISHI, 70,6, 114-120 (2014).
(2) ISO 20418-2 : 2018 Textiles – Qualitative and quantitative proteomic analysis of some animal hair fibers – Part 2: Peptide detection using MALDI-TOF MS.

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