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Application News

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MALDI-TOF Mass Spectrometry

Differentiation of Animal Hairs 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 covers the differentiation of animal hairs using the MALDI-8020, which is a benchtop MALDI-TOF MS.

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Sample Pretreatment

The raw hairs of cashmere, along with yak, sheep, and alpaca wool and camel, horse and rabbit hair were finely broken up with scissors or a ball mill. A volume of 0.5 mL of 4 % sodium dodecyl sulfate (SDS) containing 50 mM dithiothreitol (DTT) in 0.1 M phosphate buffer (pH 7.8) was added to 10 to 50 mg of the broken-up raw hair, and this sample was heated for 15 minutes to 1 hour at 95 °C. After heating, iodoacetamide was added to the extract to achieve a concentration of 100 mM, and the extract was left for 15 minutes at room temperature to allow the reaction, which was stopped by adding 10 µL of 25 mM DTT. The extract was precipitated out with trichloroacetic acid, washed, and then re-dissolved in a 7 M urea and 2 M thiourea solution. The re-dissolved extract was diluted with 50 mM ammonium bicarbonate, then 2.0 µg of trypsin was added, and this sample was digested overnight at 37 °C.

Fig. 1 MALDI-8020 Benchtop MALDI-TOF MS

Desalting was performed using ZipTip® µC18 (Merck Millipore). Following desalting, the sample solution was loaded on the MALDI target plate, 0.5 µL of matrix solution was added and mass spectrometry was performed. The matrix solution was prepared by dissolving CHCA (α-cyano-4-hydroxycinnamic acid) in 50 % acetonitrile/0.05 % trifluoracetic acid (TFA) at a concentration of 5 mg/mL.

For the MALDI analysis, a MALDI-8020 benchtop MALDI-TOF MS (Fig. 1) was used, measuring in the linear positive mode. The peak list obtained from each mass spectrum was analyzed using the eMSTAT Solution™ statistical analysis software in an attempt to identify each animal hair.

Results

Fig. 2 shows the mass spectra of trypsin-digested peptides from animal hair. Each species-specific peptide peak in the range *m/z* 2450 to 2750 was detected. By using these specific peaks, it is possible to differentiate animal hairs. The main constituent protein of animal hair is keratin, and the peptides that are detected are also derived from keratin.

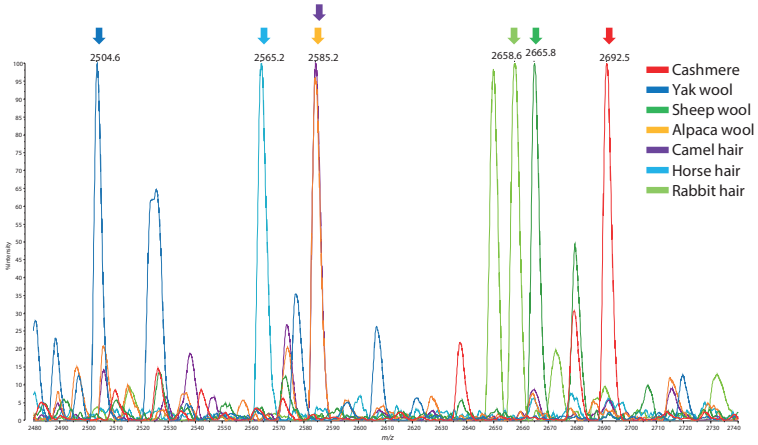

Fig. 2 Mass Spectra of Trypsin-digested Peptides from Animal Hair

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

Species of Organism	Keratin Type I Protein	Amino Acid Sequence	$[M+H]^+$ Average Mass *1
Cashmere	Keratin 33A [*Capra hircus*]	YSCQLNQVQSLIVNVESQLAEIR	2692.38
Yak	Keratin type I microfibrillar, 47.6 kDa-like [*Bos mutus*]	YSSQLAQVQGLIGNVESQLAEIR	2504.81
Sheep	Keratin 33B [*Ovis aries*]	YSCQLSQVQSLIVNVESQLAEIR	2666.03
Camel/alpaca	Keratin, type I microfibrillar, 47.6 kDa[*Camelus dromedaries*]	YGSQLSQVQGLITNVEHQLAEIR	2584.90
Horse	Keratin 33A [*Equus caballus*]	YSSQLSQVQGLITNVESQLAEIR	2564.86
Rabbit	Keratin, type I cuticular Ha3-I [*Oryctolagus cuniculus*]	YSSQLSQVQCMISNVESQLGEIR	2657.99
Dog	Keratin 33A [*Canis lupus familiaris*]	YSSQLNQVQCMITNVESQLAEIR	2713.07
Brown rat	Keratin 31 [*Rattus norvegicus*]	YSSQLSQVQCLITNVESQLGEIR	2653.98
Human	KRT34 protein [*Homo sapiens*]	YSSQLSQVQSLITNVESQLAEIR	2594.89

*1 The average mass of cysteine residues is calculated after their carbamidomethylation by iodoacetamide.

The results of attribution of animal species-specific peaks are shown in Table 1 [1)]. These amino acid sequences are derived from keratin type I. It can be seen that these sequences are specific to animal species as well as human beings and brown rats. Note that the masses of the specific peaks for alpaca and camel coincide, so that these two cannot be distinguished from each other using this method.

Subsequently, using the animal species-specific peaks shown in Fig. 2 and eMSTAT Solution, we created a discrimination model (algorithm: Support Vector Machine) to determine the animal species that hairs came from (Fig. 3 left). Using this discrimination model, we were able to determine the correct animal species upon discriminant analysis of three separately analyzed samples – of cashmere, yak wool and sheep wool – in all cases (Fig. 3 right).

Conclusion

It was shown that animal hairs could be quickly differentiated using the MALDI-8020 benchtop MALDI-TOF MS and the statistical analysis software eMSTAT Solution. By using this method, it is considered possible to make various differentiations, extending beyond cashmere to feathers, human hair, and so on. The MALDI-8020, which combines the compactness of a benchtop instrument with performance sufficient for molecular profiling, is expected to develop in the future as a product that can easily and quickly assess the properties of many kinds of samples.

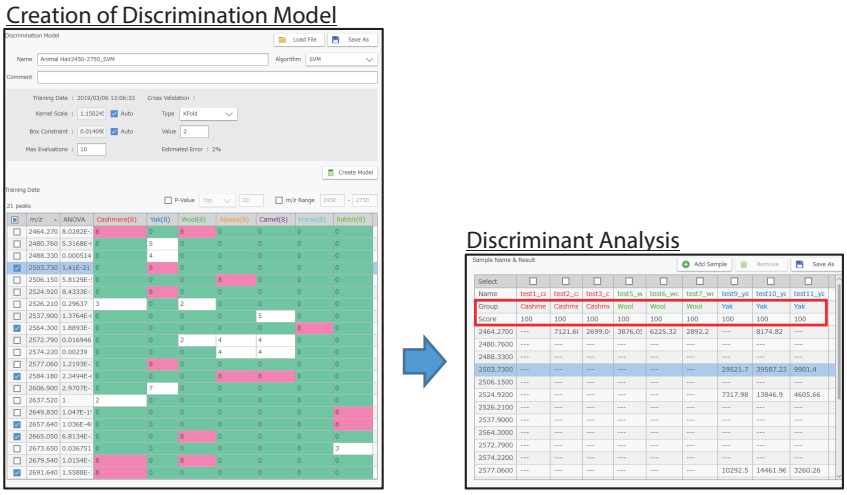

Fig. 3 Discriminant Analysis of Animal Hair (Left: Discrimination Model Created, Right: Results of Discriminant Analysis of Animal Hair)

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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