ApplicationNote

ProteoMass™ Peptide and Protein MALDI-MS Calibration Kit

By Layle Barbacci and Judy Boland
Sigma-Aldrich Corporation, St. Louis, MO USA

Introduction

The field of proteomics focuses on protein characterization in an effort to understand genomic sequence information. Proteomics involves the comprehensive study of the proteins within a cell to elucidate structure and function and how these relate to biological processes. The most common technique used in protein characterization is mass spectrometry.

Matrix assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry (MS) is a rapid and sensitive technique for the characterization of peptides and proteins. Used in a variety of modes, MALDI-MS provides information such as the molecular weight of an intact protein, peptide mass mapping from a tryptic digest, and peptide sequencing. For optimum results, frequent calibration and tuning of the MALDI mass spectrometer are necessary.

Until now, all the reagents used in tuning and calibrating a MALDI mass spectrometer were sold individually, often in much larger amounts than are required for use. Here we describe the use of the MS-CAL1 kit for instrument calibration for both low and high MW ranges and how this calibration is applied to data collection and analysis. The components of the kit provide the tools necessary for protein and peptide characterization with maximized convenience and minimal waste.

Materials and Methods

All materials were supplied by Sigma-Aldrich Corporation (St. Louis, MO) unless otherwise stated. Materials without product code listings are components of the ProteoMass Peptide and Protein MALDI-MS Calibration Kit (MS-CAL1). The products used for trypsin in-gel digest analysis are not components of the kit.

The ProteoMass Peptide and Protein Kit contains ten individual peptides and proteins packaged at 10 nanomoles per vial. This package size is convenient for both molecular weight calibration and for sensitivity testing, without the need for numerous serial dilutions. The peptides and proteins included were chosen for their purity and their ability to cover a wide mass range for calibration. Standards range from bradykinin fragment 1-7 with a mass of 757 Da to bovine serum albumin with a mass of 66,430 Da. Several low molecular weight (MW) peptides are included for calibration in the typical range (800-3,000 Da) of tryptic digestion fragments. The higher MW proteins are useful in the analysis of intact proteins. Two peptides, $P_{14}R$ and angiotensin II, are included for calibration of post source decay (PSD) fragments which provides peptide sequence information.

The matrices specially purified for MALDI-MS are packaged in amber vials at 10 mg per vial and provide consistent results over a week of usage once in solution. Both α-cyano-4-hydroxy-cinnamic acid (α-cyano) and sinapinic acid are supplied for use with the low and high molecular weight standards, respectively.

Solvents included in the kit, acetonitrile (ACN) and trifluoroacetic acid (TFA; 0.1 and 1% (v/v) solutions), are tested to ensure low alkali metal content and high purity. The solvents are packaged in high-density polyethylene (HDPE) which eliminates the leaching of sodium and potassium into the solvent, a common problem with glass bottles leading to lower resolution and cation adducts.

All the standards were tested on a Kompact SEQ and AXIMA-CFR (Shimadzu Biotech, UK) to meet certain performance criteria in selected modes of positive ion MALDI mass spectrometric analysis (linear, reflectron, or PSD). This does not preclude the use of these standards in other modes (i.e. negative ion mode) or with instruments made by other manufacturers.

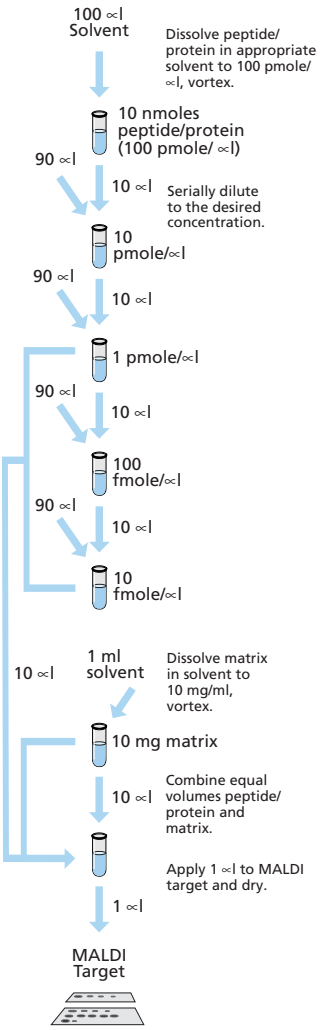

Figure 1. Protocol for Sensitivity Testing

Preparation of standard stock solutions

For a stock solution of 100 pmol/µl, the contents of each standard tube were dissolved in 100 µl of the appropriate solvent. Insulin oxidized B chain was dissolved in 50% ACN with 0.05% TFA. All other standards were dissolved in the 0.1% TFA solution. Stock solutions were stored frozen.

Preparation of solutions for sensitivity analysis

Serial dilutions of the 100 pmol/µl angiotensin II and cytochrome c stock solutions with the appropriate solvent were performed according to the Protocol for Sensitivity Testing (Figure 1) to produce working solutions with concentrations ranging between 10 fmol/µl and 10 pmol/µl.

Preparation of solutions for calibration

Beginning with the 100 pmol/µl stock solutions, a peptide calibration mixture was prepared by combining bradykinin fragment 1-7(15 µl), angiotensin II (10 µl), ACTH fragment 18-39 (5 µl), and insulin oxidized B chain (20 µl) and diluting to 100 µl with the 0.1% TFA solution (Figure 2). Peptide volumes shown in Figure 2 may need to be adjusted to give consistent signal across the mass range. The calibration solution was then diluted 1:10 (v/v) with the 0.1% TFA solution. A protein calibration solution was prepared by mixing cytochrome c (1 µl), aldolase (2 µl), and albumin (10 µl) and diluting to 100 µl with 0.1% TFA. Typical calibration solution concentrations range between 1 and 10 µM (pmol/µl) for each component. Higher concentrations of larger molecular weight species in the peptide/protein mixtures may be necessary to optimize signal intensities across the mass range of interest. For the best mass accuracy, bracket the mass range of interest and, when possible, use three to four peptides/proteins for calibration.

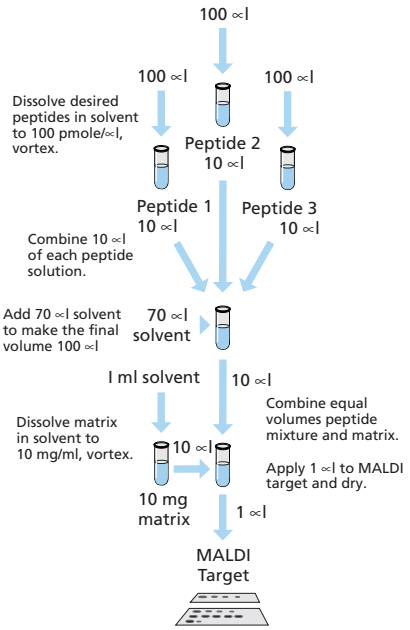

Figure 2. Protocol for Calibration

Preparation of MALDI Matrices

The contents of a 10-mg tube of α-cyano and sinapinic acid matrix were dissolved in 1 ml of the 50% ACN in 0.05% TFA solution and 70% ACN in 0.03% TFA solution, respectively. Once in solution, the matrices were stored in the dark and used for one week, then discarded. Using the 50% ACN in 0.05% TFA solvent, both α-cyano and sinapinic acid form nearly saturated solutions at room temperature. Centrifugation pellets any residual crystals in the matrix solution. The ACN concentration may be adjusted to suit individual preferences. A mixture of 70% ACN and 30% of the 0.1% TFA solution is often used.

Preparation of MALDI Samples and Target

Each standard solution was mixed 1:1 (v/v) with the appropriate matrix (α-cyano and sinapinic acid for the peptide and protein solutions, respectively) and vortexed. A 1.0-µl aliquot of standard/matrix mixture was spotted onto a clean MALDI target (Shimadzu Biotech, UK). Analysis was done in the linear positive ion mode using a Kompact SEQ mass spectrometer.

Trypsin In-gel Digest Analysis

Carbonic anhydrase II (0.5 µg, Product Code: C 6403) was separated on a 4-20% Tris-Glycine gel (Invitrogen, Carlsbad, CA). The protein spot was excised from the gel. The gel slice was placed in a tube with 20 µl of a 20 µg/ml solution of proteomics sequencing grade trypsin (Product Code: T 6567) in 40 mM ammonium bicarbonate (Product Code A 6141) containing 10% ACN (Product Code 27,071-7). An additional 50 µl of 40 mM ammonium bicarbonate with 10% ACN was added and the mixture was incubated overnight at 37 °C with agitation. The digest solution was bound to a ZipTip® pipette tip (Millipore Corporation, Bedford, MA) and eluted directly from the tip onto the MALDI target with 1.5 µl of the α-cyano matrix solution.

Results and Discussion

Analysis of the dilution series of angiotensin II and cytochrome c resulted in detection levels of 50 fmol and 500 pmol, respectively (data not shown). The mass spectrum of the peptide calibration solution is shown in Figure 3. The standards span a mass range between 757 and 3,496 Da and provide an ideal calibration solution for a tryptic digest. The mass spectrum of the protein calibration solution is shown in Figure 4. The mass range bracketed by these protein ranges from 12,362 to 66,430 Da and can be extended up to 132,859 Da if the $(2M+H)^{+1}$ peak of albumin is used. The mass spectrum of the in-gel digestion of carbonic anhydrase II is shown in Figure 5. Close external calibration was done just prior to the analysis of the digestion solution providing confidence in the molecular weight assignments of the tryptic fragments. The resulting peptide masses were searched using the Mascot search engine (www.matrixscience.com) at a tolerance of 300 ppm. The database search resulted in carbonic anhydrase II as the top assignment with 42% sequence coverage and a MOWSE score of 102.

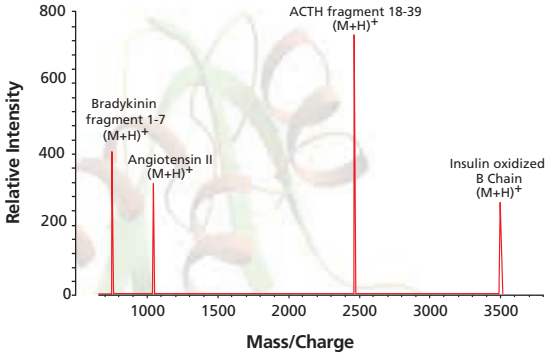

Figure 3. *MALDI mass spectrum of a peptide calibration solution.*

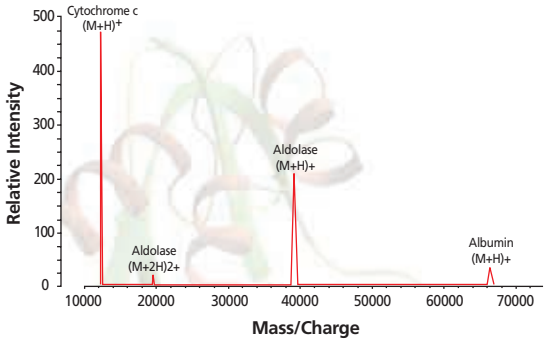

Figure 4. *MALDI mass spectrum of a protein calibration solution.*

Conclusions

Sigma-Aldrich has developed three kits to suit the needs of peptide and protein researchers. MS-CAL1 includes both peptides and proteins with α-cyano and sinapinic acid for the greatest versatility. MS-CAL2 includes only peptides (two vials each of five peptides) and α-cyano matrix. MS-CAL3 includes only the proteins (two vials each of five proteins) and the sinapinic acid. Our kits eliminate the need to purchase multiple components individually and reduce waste. Additionally, the kits include standards, specially purified matrices, and low-alkali metal solvents resulting in cleaner, more simplified mass spectra.

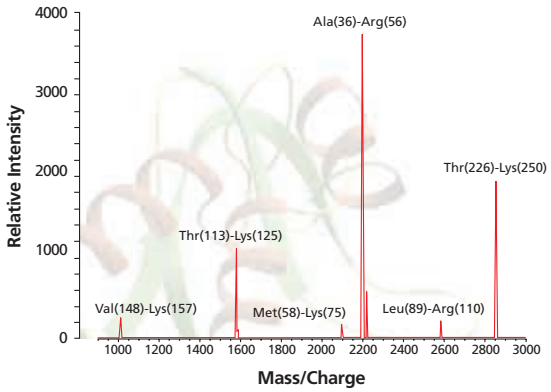

Figure 5. *MALDI mass spectrum of an in-gel digestion of carbonic anhydrase II.*

About the Authors

Layle Barbacci, Ph.D., and Judy Boland, M.S., are Senior Scientists in the Biotechnology R&D Department of Sigma-Aldrich, St. Louis, MO.

ORDERING INFORMATION

Product Code	Mass Range	Standards	Solvents	Matrices	Application
MS-CAL1	757 Da to 66,430 Da	Proteins & Peptides	X	X	Excellent for evaluating complex mixtures of proteins and peptides that span a broad range of molecular weights.
MS-CAL2	757 Da to 3,494 Da	Peptides	X	X	Provides accurate calibration over the typical mass range of tryptic digestion fragments in reflection mode. Superior performance in either internal or external calibration.
MS-CAL3	5,730 Da to 66,430 Da	Proteins	X	X	Enables precise calibration over the typical protein mass range in either internal or external applications.