Determination of protein synthesis in vivo using labeling from deuterated water and analysis of MALDI-TOF spectrum

Gary Guishan Xiao,1 Meena Garg,1 Shu Lim,1 Derek Wong,1 Vay Liang Go,2 and Wai-Nang Paul Lee1
1Department of Pediatrics, Division of Endocrinology, Los Angeles Biomedical Research Institute at Harbor-University of California Los Angeles Medical Center; and 2Department of Medicine, Division of Gastroenterology, David Geffen School of Medicine, Los Angeles, California

Submitted 14 September 2007; accepted in final form 12 December 2007

Determination of protein synthesis in vivo using labeling from deuterated water and analysis of MALDI-TOF spectrum. J Appl Physiol 104: 828–836, 2008. First published January 10, 2008; doi:10.1152/japplphysiol.00976.2007.—This paper describes a method of determining protein synthesis and turnover in vivo labeling of protein with deuterated water and analysis of matrix-assisted laser desorption time-of-flight mass spectrometer (MALDI-TOF) spectrum. Protein synthesis is calculated using mass isotopomer distribution analysis instead of precursor to product amino acid enrichment ratio. During protein synthesis, the incorporation of deuterium from water changes the mass isotopomer distribution (isotope envelop) according to the number of deuterium atoms (0, 1, 2, 3, etc.) incorporated, and the distribution of the protein with 0, 1, 2, 3,… atoms of deuterium follows a binomial distribution. A mathematical algorithm by which the distribution of deuterium isotopomers can be extracted from the observed MALDI-TOF spectrum is presented. Since deuterium isotopomers are unique to newly synthesized proteins, the quantitation of their distribution provides a method for the quantitation of newly synthesized proteins. The combined use of postsource decay sequence identification and mass isotopomer distribution analysis makes the use of in vivo labeling with deuterated water a precise method to determine specific protein synthesis.

Protein synthesis rate (PSR) is calculated by dividing the quantity of new protein by the time interval for the change using the equation:

\[
PSR = \frac{[\text{protein concentration}] \times (\text{FNP})}{(\text{unit time})}
\]

Examples of such applications for the determination of protein synthesis and turnover using tracers have been reviewed by Wolfe (21).

Recently, Previs and colleagues (16) and Hellerstein and colleagues (3) separately introduced a novel method for the determination of protein synthesis using in vivo labeling with deuterated water (D2O) or heavy water. When cells or animals are given deuterium water, it is possible to maintain a constant high level of enrichment in water (0.5–2%). Plasma alanine rapidly incorporates the deuterium from water, changing its CH3- group to CD3-, thus increasing its molecular weight depending on the deuterium enrichment in water (21). The enrichment in alanine can be determined by gas chromatography/mass spectrometry (GC/MS) after derivatization. To determine protein synthesis, the specific protein such as albumin is first isolated and hydrolyzed, and the enrichment in alanine isolated from the protein is similarly determined by GC/MS. FNP can be calculated using Eq. 1. The method of Hellerstein and colleagues (3) differs from the method of Previs and colleagues (16) in that a different experimental approach was used to determine amino acid enrichment using mass isotopomer distribution analysis (MIDA). It should be noted that these methods are variations of the basic precursor/product enrichment ratio method. They all require 1) determination of precursor SA or E, 2) isolation of specific protein of interest, 3) hydrolysis of the protein to separate the amino acids, 4) determining the enrichment in the specific amino acid of interest, and 5) application of Eqs. 1 and 2. A major disadvantage of this approach is that the accuracy of the calculated protein synthesis depends on the purity of the protein isolated.

We describe here a novel approach for the determination of protein synthesis and turnover using 2H-labeling and protein
mass spectrum. It is known that metabolic systems in cells of organisms are capable of synthesizing nonessential amino acids. In the process of amino acid synthesis, carbon, nitrogen, and hydrogen atoms are incorporated from their precursor substrates either directly or through exchanges (transamination). If these precursor substrates are enriched with one or more of these isotopes, the resultant amino acids are heavier than the “natural” amino acids. Protein molecules that are synthesized after the introduction of the heavy isotopes will contain the heavier amino acids, resulting in a mass shift in the corresponding spectrum (Fig. 1). The intensities of the mass peaks corresponding to the unlabeled (light) and the labeled (heavy) can be used for calculation of the newly synthesized fraction. The method described herein differs from previous methods in that it utilizes the intensities of all relevant mass peaks of the peptide spectrum instead of the intensities of unique mass peaks. The mass spectral peaks of the heavier protein are resolved from the lighter (natural) protein by a mathematical algorithm. The capability of resolving overlapping spectra makes it possible to label proteins with lower enrichment of stable isotopes. The need for highly enriched precursor substrates is obviated (22).

**MATHEMATICAL ALGORITHM**

**Binomial distribution model of mass isotopomers in peptide.**

To be able to separate the “light” spectrum from that of the “heavy” or labeled spectrum, one has to fully understand the theory that governs the mass isotopomer distribution of a large molecule such as a peptide and how the incorporation of heavy isotope subsequently modifies such a distribution. Mass isotopomer distribution in a peptide spectrum is mostly generated from the presence of $^{13}$C at its natural abundance (see below for a more general discussion of isotopes in protein). The distribution of isotopomers in the “isotope envelop” can be modeled by a binomial distribution with parameters $N$ and $p$. In the case of $^{13}$C distribution in peptide, $N$ is the number of carbon atoms, and $p$ is the natural enrichment of $^{13}$C. Assum-

---

1. There are 2 parameters in a binomial distribution: the number of trials ($N$) and the probability of success ($p$). In the case of $^{13}$C distribution in peptide, $N$ is the number of carbon atoms, and $p$ is the natural enrichment. The natural abundance of $^{15}$N and $^{18}$O affects the accuracy of determining the number of carbon atoms in the peptide but not the overall isotopomer distribution in the isotope envelop.
is the molar fraction of peptide with $^{13}$C (11). The use of this model to simulate mass isotopomer distribution in the isotope envelop is shown in the following example of a peptide [mass/charge (m/z) 1393] from AMP deaminase in an albumin fraction after tryptic digest (Fig. 2). The intensities of the individual mass peaks were first expressed as a (molar) fraction of the all the isotopomer species. The theoretical distribution was calculated based on $N = 69$ and $p = 0.011$. The goodness of fit can be seen by inspection of Fig. 2 and tested by multiple linear regression analysis, which showed a coefficient of 0.96 and a $R^2$ of 0.99.

**Concatenation function ($\oplus$) of mass isotopomers.** In a molecule consisting of carbon, nitrogen, oxygen and hydrogen, the molecular weight as well as the distribution of isotopomers can be predicted from the molecular weights of the elements as well as the frequency of finding their corresponding heavy isotopes (19, 24). In general, the isotopomer distribution can be seen as a concatenation of groups of isotopomers from the same elements

[$\text{carbon isotopomers}$ $\oplus$ [$\text{hydrogen isotopomers}$]

$\oplus$ [$\text{oxygen isotopomers}$ $\oplus$ [$\text{nitrogen isotopomers}$]

$\oplus$ [$\text{sulfur isotopomers}$]

For the purpose of this paper, we simplify the experimental model to the specific condition involving deuterium:

[$\text{carbon isotopomers}$ $\oplus$ [$\text{deuterium isotopomers}$]

where $\oplus$ is the concatenation operation, which generates the mass isotopomers of the whole molecules from the isotopomers of its component parts (Fig. 3) (11). For example, if mass isotopomer distribution in carbon is given by $[a_0 m_0, a_1 m_1, a_2 m_2, \ldots ]$ and the isotopomers from deuterium is $[b_0 m_0, b_1 m_1, b_2 m_2, \ldots ]$, the isotopomers of the peptide that contains both components is given by $[a_0 b_0 m_0, (a_0 b_1 + a_1 b_0) m_1, (a_1 b_1 + a_2 b_0 + b_2 a_0) m_2, \ldots ]$. The sum of the coefficients for each component is equal to 1 ($\sum a_i = 1$; and $\sum b_i = 1$). As illustrated by the example, the coefficient of the combined peptide is given by the sum of the products with subscript indexes equal to the mass isotopomer number. In this case, the coefficient of $m_0$ is given by $a_0 b_0$ and $m_1$ is given by $a_0 b_1 + b_2 a_1$. In fact, the binomial distribution is mathematically a special case of the concatenation function.

Figure 4 shows a hypothetical example of deuterium incorporation from deuterated water into a peptide. The original peptide is represented by carbon isotopomers. When such a peptide is synthesized in the presence of deuterated water, each peptide molecule may incorporate 1, 2, or 3 deuterium atoms, depending on the enrichment in water. The final distribution of the heavy peptide is the result of concatenation of carbon-isotopomers and the deuterium isotopomers. In this example the distribution of carbon isotopomers is given by the binomial distribution with $N(c) = 100$ and $p(c) = 0.011$, and the deuterium isotopomer distribution is given by $N(d) = 20$ and $p(d) = 0.04$. These distributions are shown in Fig. 4, A and B. Using the concatenation operation on these two distributions, we arrived at the distribution of the combined distribution (see APPENDIX). Figure 4C shows the isotopomer distribution of the resultant peptide as observed in the mass.

**Concatenation Operation $\oplus$**

\[
\text{C-isotopomers} \oplus \text{d-isotopomers} \rightarrow \text{Isotopomers of peptide}
\]

**Inverse Concatenation Operation $\ominus$**

\[
\text{Isotopomers of peptide} \ominus \text{C-isotopomers} \rightarrow \text{d-Isotopomers}
\]
spectrum of the peptide. Figure 4D shows the changes in isotopomer distribution (mass spectrum) of the same peptide under 2% and 4% deuterium enrichment conditions.

The mathematical algorithm of the inverse operation (\(\mathcal{O}\)) takes the observed spectrum and the unlabeled spectrum and converts it to a deuterium isotopomer spectrum using regression analysis. It can be shown that the \(\mathcal{O}\) operation on a peptide without deuterium label (pre-existing protein) returns the result

\[ m_0 = 1 \text{ or unlabeled}^4. \]

Since \(m_0\) of the deuterium spectrum can come from the newly synthesized peptide or the preexisting peptide, the deuterium isotopomers of the deuterated protein is diluted by \(m_0\) contributed by the unlabeled protein. The ratio between the observed and the theoretical molar fractions of \(m_1, m_2, \ldots\) from the deuterium isotopomers of the newly synthesized peptide give the fraction of newly synthesized peptide (11). (Fig. 5).

**MATERIALS AND METHODS**

**Animal protocol.** The animal study was carried out according to a protocol approved by Animal Research Committee at UCLA. Sprague-Dawley rats (250 g) were treated with a priming dose of D2O equal to 6% body water and maintained on drinking water enriched with 4% deuterium as previously described (10). Serum was obtained before and on the 8th day of deuterated water treatment. Serum albumin was purified from these samples using AffiGel-Blue affinity column. The albumin fraction was eluted with 1.4 M NaCl in 20 mM Tris buffer (pH 7.1). Protein concentration in the albumin fraction was determined by its optical density (OD280). Pretreatment sample and posttreatment samples were mixed in the proper proportions of protein concentrations to simulate 10, 25, 50, and 75% synthesis. The proteins in these samples were precipitated using 10% trichloroacetic acid. The desalted sample was concentrated before trypsin digestion and analysis by mass spectrometer.

---

4 [carbon isotopomers] \(\mathcal{O}\) [carbon isotopomers] returns a coefficient for \(m_0 = 1\), and for other isotopomers, coefficients = 0.
Innovative Methodology

Acquisition of mass spectra by MALDI-TOF. Trypsin digestion was performed by following the standard protocol (17, 23) with minor modification. The purified albumin fractions were prepared in 50 mM NH4HCO3. A 50-ml aliquot was removed and mixed with 50 ml of ACN to improve proteolysis (17). Trypsin (Trypsin Gold, Promega) was added to a final protease-to-protein ratio of 1:80, and the digestion mixture was incubated at 37°C for 24 h. The digested peptides were lyophilized and resuspended in 50% acetonitrile/0.3% trifluoroacetic acid (TFA), and purified by using C18 zip tips (Millipore) before MALDI-TOF profiling and postsource decay sequence identification using a Voyager DE (Voyager-DE workstation, Applied Biosystems, Forster, CA) (23).

The purified peptides using C18 zip tip was directly eluted to MALDI plate with 2 μl of 10 mg/ml a-cyano-4-hydroxyquinamic acid (CHCA), and allowed to dry. The molecular weight of each sample was measured on a MALDI mass spectrometer with positive mode using Voyager DE reflector mode MALDI-time-of-flight (TOF). The instrument was calibrated with insulin standard (ProtoMass Insulin MALDI-MS standard [5,729,6089] and ACTH standard (ProteoMass ACTH fragment 18–39 MALDI-MS standard [2,464,1989]) (Sigma, St. Louis, MO). The mean molecular mass was calculated from multiple independent measurements. For the extraction of the protein from the MALDI plate, high laser power was applied (2,175 vs. 1,970 U for the standards). The instrument operated in positive ion delayed extraction mode with a low-mass gate of 500 Da. The accelerating voltage was 25,000 V (nondigested insulin and glucagons) or 20,000 V (trypsin-digested insulin and glucagons), the grid voltage was set to 65%, and the delayed time is 150 ns (trypsin-digested insulin and glucagons) or 285 ns (nondigested insulin and glucagons). Each spectrum was the average of ~200 scans, calibrated using external ACTH and insulin calibration files generated during the experiment.

Identification of tryptic digest by postsource decay. Tryptic digest of rat albumin was identified using postsource decay (PSD) matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry (6, 7). PSD fragment ion spectra were acquired for several peptides and peptide derivatives after isolation of the appropriate precursor ion by using timed ion selection. Fragment ions were refocused onto the final detector by stepping the voltage applied to the reflectron in the following ratios: 1.0000 (precursor ion segment), 0.9126, 0.6049, 0.4125, 0.2738, 0.1975, and 0.1213 (fragment segments). The individual segments were stitched together by using software provided by Applied Biosystems. The MS data from both tandem mass spectra from the LC-MS/MS experiments and the MALDI-MS PSD mass spectra were searched against a subset of rodent proteins in the MSDB protein sequence data base, using the Mascot software provided by Applied Biosystems. The MS data from both masses were searched against a subset of proteins in the MSDB protein sequence data base, using the Mascot software provided by Applied Biosystems. The MS data from both masses were searched against a subset of proteins in the MSDB protein sequence data base, using the Mascot software provided by Applied Biosystems. The MS data from both masses were searched against a subset of proteins in the MSDB protein sequence data base, using the Mascot software provided by Applied Biosystems. The MS data from both masses were searched against a subset of proteins in the MSDB protein sequence data base, using the Mascot software provided by Applied Biosystems. The MS data from both masses were searched against a subset of proteins in the MSDB protein sequence data base, using the Mascot software provided by Applied Biosystems.

RESULTS

Serum was collected from a Sprague-Dawley rat after 8 days of deuterated water treatment. Albumin fraction was purified by AffiGelBlue affinity column. After tryptic digest, the sample was analyzed by MALDI-TOF for peptides in the range of 1,000–2,000 Da. Five peptides were identified using MALDI-TOF and postsource decay analysis. Their sequences were matched to rat serum albumin, AMP deaminase, and alpha 2u-globulin (Table 1). Despite our attempt to purify albumin, the MALDI-TOF mass spectrometry indicated contamination of albumin by other serum proteins. The corresponding normalized spectra of these peaks from the sample before deuterated water treatment (unlabeled spectra) are shown in Table 2. Average mass was calculated using method of Blom (2).

Table 1. Peptides identified with MALDI-TOF PSD

<table>
<thead>
<tr>
<th>m/z</th>
<th>Sequences</th>
<th>Protein Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,299.4</td>
<td>HPDYSVSLLLR</td>
<td>Rat serum albumin</td>
</tr>
<tr>
<td>1,393.4</td>
<td>MLPFKLTEIDDM</td>
<td>Rat AMP deaminase</td>
</tr>
<tr>
<td>1,479.5</td>
<td>LGEYSPQNAIYR</td>
<td>Rat serum albumin</td>
</tr>
<tr>
<td>1,609.4</td>
<td>DVFGLGFLEYSK</td>
<td>Rat albumin</td>
</tr>
<tr>
<td>1,681.7</td>
<td>IRENGSMR</td>
<td>Rat alpha2u-globulin</td>
</tr>
</tbody>
</table>

Tryptic digest of rat albumin was identified using postsource decay (PSD) matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry (6, 7). m/z, mass to charge ratio.

Table 2 shows the normalized spectra of peptides isolated from serum after 8 days of deuterated water treatment. The incorporation of deuterium in these peptides results in small shifts in the average mass. The magnitude of the shift due to deuterium incorporation depends on the enrichment in water, the number of nonessential amino acids, and the fraction of new protein. The fraction of new protein was calculated using the inverse concatenation operation. Examples of such a calculation are shown in the Appendix. Since the operation utilizes information from all spectral peaks, an estimate of the accuracy (SD) can be obtained from a single spectrum in m/z 1,681. From the magnitude of mass shift and the calculated new fraction, the number of possible deuterium substitution [N(d)] can be estimated.5 It is to be expected that peptides of the same protein have the same synthesis rate and new fraction, which is shown in the albumin peptides in Table 2. Peptides from other proteins may have different synthesis rates and have different new fractions.

The fraction of new protein can also be determined from the peptide spectrum when the spectra of the labeled and unlabeled peptides are known. In the example of AMP deaminase, we found that the protein was almost completely labeled (100% new synthesis) by day 8 of deuterated water treatment. Protein fractions containing albumin and AMP deaminase from sera obtained before deuterium labeling and on day 8 after deuterium labeling were mixed in to simulate 10, 25, 50, and 75% synthesis. The different mixtures were subject to trypsin digest and MALDI-TOF analysis. The observed spectra are shown in Table 4. Using multiple linear regression analysis, we determined the coefficients for labeled and unlabeled peptides that would give the observed distributions. The coefficient representing percent synthesis and its SD were provided by the regression analysis (Table 4). Linear response of isotopomer distribution due to mixing of labeled and unlabeled peptides is shown in Fig. 6. As expected, the SD for each measurement increases as percent synthesis decreases. Therefore, protein synthesis and protein concentration can be determined from proteins that are maximally enriched (i.e., 100% newly synthesized).

DISCUSSION

Mass isotopomer distribution analysis has been used to determine protein synthesis and turnover. Specifically labeled

5 N(d) is the theoretical number of deuterium atoms incorporated when deuterated water enrichment is 100%.
Determination of Protein Synthesis Using MIDA

Table 2. Mass isotopomer distributions of the identified peptides before deuterium labeling

<table>
<thead>
<tr>
<th>m/z</th>
<th>m/z 1,299</th>
<th>m/z 1,393</th>
<th>m/z 1,479</th>
<th>m/z 1,609</th>
<th>m/z 1,681</th>
</tr>
</thead>
<tbody>
<tr>
<td>m0</td>
<td>0.4892 ± 0.0038</td>
<td>0.4466 ± 0.0101</td>
<td>0.4239 ± 0.0029</td>
<td>0.3802 ± 0.0016</td>
<td>0.3307</td>
</tr>
<tr>
<td>m1</td>
<td>0.3418 ± 0.0074</td>
<td>0.3393 ± 0.0047</td>
<td>0.3349 ± 0.0056</td>
<td>0.3343 ± 0.0022</td>
<td>0.3120</td>
</tr>
<tr>
<td>m2</td>
<td>0.1250 ± 0.0089</td>
<td>0.1448 ± 0.0024</td>
<td>0.1480 ± 0.0030</td>
<td>0.1807 ± 0.0034</td>
<td>0.1880</td>
</tr>
<tr>
<td>m3</td>
<td>0.0368 ± 0.0010</td>
<td>0.0474 ± 0.0110</td>
<td>0.0581 ± 0.0056</td>
<td>0.0724 ± 0.0010</td>
<td>0.0913</td>
</tr>
<tr>
<td>m4</td>
<td>0.0060 ± 0.0039</td>
<td>0.0127 ± 0.0044</td>
<td>0.0247 ± 0.0025</td>
<td>0.0217 ± 0.0017</td>
<td>0.0430</td>
</tr>
<tr>
<td>m5</td>
<td>0.0007 ± 0.0004</td>
<td>0.0051 ± 0.0025</td>
<td>0.0105 ± 0.0038</td>
<td>0.0106 ± 0.0023</td>
<td>0.0156</td>
</tr>
<tr>
<td>Average mass</td>
<td>1,300.1348</td>
<td>1,394.2474</td>
<td>1,479.4711</td>
<td>1,610.4081</td>
<td>1,682.8889</td>
</tr>
</tbody>
</table>

The averages and SD were calculated from triplicate MALDI-TOF analyses. We had only 1 analysis for the peak at m/z 1.681. Average mass was calculated according to Blom (2). m0, molar fraction of monoisotopic species; m1, m2, m3, m4, and m5 are molar fraction of isotopomer molecules with 1, 2, 3, 4, and 5 isotopic substitutions, respectively.

Table 3. Mass isotopomer distributions of the identified peptides after deuterium labeling

<table>
<thead>
<tr>
<th>m/z</th>
<th>m/z 1,299</th>
<th>m/z 1,393</th>
<th>m/z 1,479</th>
<th>m/z 1,609</th>
<th>m/z 1,681</th>
</tr>
</thead>
<tbody>
<tr>
<td>m0</td>
<td>0.3409 ± 0.0089</td>
<td>0.2748 ± 0.0044</td>
<td>0.2672 ± 0.0073</td>
<td>0.2757 ± 0.0026</td>
<td>0.2216</td>
</tr>
<tr>
<td>m1</td>
<td>0.3418 ± 0.0061</td>
<td>0.3392 ± 0.0090</td>
<td>0.3159 ± 0.0127</td>
<td>0.3266 ± 0.0120</td>
<td>0.2902</td>
</tr>
<tr>
<td>m2</td>
<td>0.1931 ± 0.0084</td>
<td>0.2205 ± 0.0063</td>
<td>0.2089 ± 0.0023</td>
<td>0.2266 ± 0.0028</td>
<td>0.2343</td>
</tr>
<tr>
<td>m3</td>
<td>0.0726 ± 0.0110</td>
<td>0.1025 ± 0.0028</td>
<td>0.1201 ± 0.0073</td>
<td>0.1082 ± 0.0057</td>
<td>0.1325</td>
</tr>
<tr>
<td>m4</td>
<td>0.0192 ± 0.0023</td>
<td>0.0395 ± 0.0014</td>
<td>0.0550 ± 0.0030</td>
<td>0.0451 ± 0.0044</td>
<td>0.0652</td>
</tr>
<tr>
<td>m5</td>
<td>0.0133 ± 0.0056</td>
<td>0.0160 ± 0.0022</td>
<td>0.0329 ± 0.0072</td>
<td>0.0178 ± 0.0042</td>
<td>0.0284</td>
</tr>
<tr>
<td>Average mass</td>
<td>1,300.4942</td>
<td>1,394.7257</td>
<td>1,480.9391</td>
<td>1,610.7292</td>
<td>1,683.2361</td>
</tr>
<tr>
<td>ΔMass</td>
<td>0.3594</td>
<td>0.4783</td>
<td>0.522</td>
<td>0.3211</td>
<td>0.3472</td>
</tr>
<tr>
<td>N(d)</td>
<td>21</td>
<td>20</td>
<td>30</td>
<td>17</td>
<td>22</td>
</tr>
<tr>
<td>New fraction</td>
<td>0.6960 ± 0.0827</td>
<td>0.6918 ± 0.0158</td>
<td>0.6994 ± 0.0442</td>
<td>0.7380 ± 0.0131</td>
<td>0.6326 ± 0.0602</td>
</tr>
</tbody>
</table>

The averages and SD were calculated from triplicate MALDI-TOF analyses. We had only 1 analysis for the peak at m/z 1.681. N(d), p, and q are defined in APPENDIX. The observed mass isotopomer distributions were first converted to their corresponding deuterium isotopomer distribution (d-isotopomers) using the inverse concatenation function. The new fractions were then calculated as in the Appendix. Average mass was calculated according to Blom (2). The change in average mass is given by N(d) × p × (new fraction).
Innovative Methodology

Determination of Protein Synthesis Using MIDA

Table 4. Determination of percent synthesis using “labeled” and “unlabeled” spectra and multiple linear regression analysis

<table>
<thead>
<tr>
<th></th>
<th>Unlabeled</th>
<th>10% Labeled</th>
<th>25% Labeled</th>
<th>50% Labeled</th>
<th>75% Labeled</th>
<th>100% Labeled</th>
</tr>
</thead>
<tbody>
<tr>
<td>m0</td>
<td>0.4466 ± 0.0101</td>
<td>0.4333 ± 0.0072</td>
<td>0.4061 ± 0.0106</td>
<td>0.3550 ± 0.0106</td>
<td>0.3054 ± 0.0106</td>
<td>0.2748 ± 0.0044</td>
</tr>
<tr>
<td>m1</td>
<td>0.3393 ± 0.0047</td>
<td>0.3402 ± 0.0036</td>
<td>0.3360 ± 0.0222</td>
<td>0.3280 ± 0.0106</td>
<td>0.3247 ± 0.0106</td>
<td>0.3392 ± 0.0090</td>
</tr>
<tr>
<td>m2</td>
<td>0.1448 ± 0.0024</td>
<td>0.1574 ± 0.0014</td>
<td>0.1667 ± 0.0082</td>
<td>0.1813 ± 0.0106</td>
<td>0.1963 ± 0.0106</td>
<td>0.2205 ± 0.0063</td>
</tr>
<tr>
<td>m3</td>
<td>0.0474 ± 0.0110</td>
<td>0.0549 ± 0.0021</td>
<td>0.0576 ± 0.0037</td>
<td>0.0774 ± 0.0106</td>
<td>0.0958 ± 0.0106</td>
<td>0.1025 ± 0.0028</td>
</tr>
<tr>
<td>m4</td>
<td>0.0127 ± 0.0044</td>
<td>0.0095 ± 0.0038</td>
<td>0.0179 ± 0.0026</td>
<td>0.0309 ± 0.0106</td>
<td>0.0459 ± 0.0106</td>
<td>0.0395 ± 0.0014</td>
</tr>
<tr>
<td>m5</td>
<td>0.0051 ± 0.0025</td>
<td>0.0047 ± 0.0029</td>
<td>0.0157 ± 0.0094</td>
<td>0.0208 ± 0.0106</td>
<td>0.0249 ± 0.0106</td>
<td>0.0160 ± 0.0022</td>
</tr>
<tr>
<td>%Synthesis</td>
<td>15.4 ± 6.8</td>
<td>25.6 ± 7.1</td>
<td>47.8 ± 3.5</td>
<td>68.6 ± 1.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Protein fractions containing albumin and AMP deaminase from sera obtained before deuterium labeling and on day 8 after deuterium labeling were mixed in to simulate 10, 25, 50, and 75% synthesis. The averages and SD of peptide m0; 1,393 were calculated from triplicate MALDI-TOF analyses for the different mixtures.

determine protein synthesis. The use of stable isotopes in the quantitation of proteins has been reviewed (1, 12, 20). These methods are used in the determination of \( \frac{J}{\text{expression in proteomics.}} \) protein identification, and \( \frac{3}{\text{protein turnover (synthesis)}} \) protein synthesis or quantitation. The calculated percent syntheses are plotted against the expected labeled ratios from Table 4. The linearity of the mass isotopomer analysis using multiple linear regression analysis is demonstrated. A useful approach in the studies of protein turnover and protein expression in proteomics.

**APPENDIX**

The concatenation operation (\( \oplus \)) of mass isotopomers. In a molecule that contains more than one element containing isotope species, the molecular spectrum can be considered as a complex mixture of its components. For example, the distribution of molecules affected by \( ^{13}C \) natural abundance is approximated by a binomial distribution with \( N(c) \) being the number of carbon and \( p(c) \) being the natural enrichment (1.1%). This distribution is reflected in the isotopomer distribution reflected in the isotope envelop. In a protein that is synthesized in the presence of deuterium (e.g., 4.0% deuterated water), when the contribution of other isotopes is ignored, the distribution of molecules having 0, 1, 2, 3, … deuterium substitutions is given by the binomial distribution with \( N(d) \) being the number of possible deuterium substitution and \( p(d) \) the deuterium enrichment in water. The isotopomer distribution in these components is designated as (C-isotopomers) and (d-isotopomers). The protein spectrum can be considered as the weighted sum of a series of spectra having 0, 1, 2, 3, … deuterium substitutions, which can be derived from (C-isotopomers) and (d-isotopomers) using the concatenation operation (\( \oplus \)).

\[
\text{(C-isotopomers)} \oplus \text{(d-isotopomers)} = \text{observed mass isotopomers in protein.}
\]

If distribution of isotopomer in (C-isotopomers) is represented by \( (a_0b_0m_0, a_1b_1m_1, a_2b_2m_2, \ldots) \) and distribution of isotopomer in (d-isotopomers) by \( (b_0m_0, b_1m_1, b_2m_2, \ldots) \), the distribution in the protein or peptide is given by \( [a_0b_0m_0, (a_0b_1 + a_1b_0)m_1, (a_0b_2 + a_1b_1 + a_2b_0)m_2, \ldots] \), where \( m_0 \) represents the monoisotopic species and \( m_1, m_2, m_3, \ldots \), are the isotopomers containing 1, 2, 3, … \( ^{13}C \) or \( ^{2}H \)

Table 5. Results from multiple linear regression analysis using an Excel spreadsheet program

<table>
<thead>
<tr>
<th>Intercept</th>
<th>Coefficients</th>
<th>SE</th>
<th>t Statistic</th>
<th>P Value</th>
<th>Predicted Coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>X Variable 1</td>
<td>0.6154</td>
<td>0.0300</td>
<td>0.0030</td>
<td>205.5862</td>
<td>0.6027</td>
</tr>
<tr>
<td>X Variable 2</td>
<td>0.2919</td>
<td>0.0038</td>
<td>77.6582</td>
<td>0.0082</td>
<td>0.3091</td>
</tr>
<tr>
<td>X Variable 3</td>
<td>0.0727</td>
<td>0.0038</td>
<td>18.9608</td>
<td>0.0335</td>
<td>0.0753</td>
</tr>
<tr>
<td>X Variable 4</td>
<td>0.0138</td>
<td>0.0038</td>
<td>3.6419</td>
<td>0.1706</td>
<td>0.0116</td>
</tr>
<tr>
<td>X Variable 5</td>
<td>0.0078</td>
<td>0.0031</td>
<td>2.5213</td>
<td>0.2404</td>
<td>0.0013</td>
</tr>
</tbody>
</table>

Using the ratio of consecutive isotopomers relationship that \( m_2/m_1 = \frac{N(d) - 1}{2} \times \frac{1}{p(1 - p)} \), we determined the theoretical \( N(d) \) to be 20. The theoretical deuterium isotopomers of (20, 0.025) are shown in the last column corresponding to fractions of molecules with 0, 1, 2, 3, and 4 deuterium substitution. The coefficients for m0, m1, m2, … are represented by X variable 1, X variable 2, etc.

Fig. 6. Linearity of mass isotopomer analysis for the determination of protein synthesis or quantitation. The calculated percent syntheses are plotted against the expected labeled ratios from Table 4. The linearity of the mass isotopomer analysis using multiple linear regression analysis is demonstrated.

\[
y = 0.941x \\
R^2 = 0.9744
\]
isotopes. The use of $m_i$ notation allows the comparison of peptide peaks of different masses.

(C-isotopomers) distribution is given by $(a_0, a_1, a_2, a_3, \ldots)$

(d-isotopomer) distribution is given by $(b_0, b_1, b_2, b_3, \ldots)$

isotopomers of the protein is given by $(c_0, c_1, c_2, c_3, \ldots)$

$(a_0, a_1, a_2, a_3, \ldots) \oplus (b_0, b_1, b_2, b_3, \ldots) = (c_0, c_1, c_2, c_3, \ldots)$

The concatenation operation is defined by the matrix multiplication below. The C-isotopomers are arranged in a $(n+1$ by $m+1$) matrix, and the d-isotopomers by a $(m+1$ by $1$) column vector. The product is the C-isotopomers in the form of a $(n+1$ by $1$) column vector:

\[
\begin{bmatrix}
 a_0 & a_1 & a_2 & \cdots & a_n \\
 b_0 & b_1 & b_2 & \cdots & b_n \\
 \vdots & \vdots & \vdots & \ddots & \vdots \\
 a_{n-1} & a_{n-2} & \cdots & a_0 & b_n \\
\end{bmatrix} \begin{bmatrix}
 c_0 \\
 c_1 \\
 \vdots \\
 c_n \\
\end{bmatrix}
\]

If the number of C-isotopomers is given by $x$, the number of C-isotopomers ($x$) is less than or equal to the number of observed isotopomers in the spectrum ($n > x$ and $n > m$). The value of $a_i$ is 0 for $n > i$.

For a mass isotopomer distribution $c_i$ with $i = n$, the first matrix has the dimension of $(n + 1$ by $m + 1$) of the C-isotopomers and the second matrix is a column matrix ($m + 1$ by $1$) of the d-isotopomers. Many of the elements of these matrices may be zero as discussed above. The resultant (C-isotopomers) distribution is the product of these two matrices. Thus

\[
c_0 = a_0 b_0 \\
c_1 = a_0 b_1 + a_1 b_0 \\
c_2 = a_0 b_2 + a_1 b_1 + a_2 b_0 \\
c_3 = a_0 b_3 + a_1 b_2 + a_2 b_1 + a_3 b_0 \\
\]

The inverse operation $\ominus$ of the concatenation operation is the inverse of the above algorithm, which is to determine the d-isotopomer distribution given column matrix of observed-isotopomers and square matrix of the C-isotopomers.

\[
(c_0, c_1, c_2, c_3, \ldots) \ominus (a_0, a_1, a_2, a_3, \ldots) = (b_0, b_1, b_2, b_3, \ldots)
\]

It is clear by inspection that d-isotopomer distribution can be determined using multiple linear regression analysis. The coefficients obtained from linear multiple regression analysis is the deuteron isotopomer distribution $(b_0, b_1, b_2, b_3, \ldots)$.

Using a binomial distribution with 100 carbons and $^{13}C$ enrichment of 0.011, we can show the (C-isotopomer distribution) generated for a peptide is $(0.3308, 0.3680, 0.2026, 0.0736, 0.0199, 0.0042)$. This is the isotope envelop below deuteron incorporation. Similarly, using a binomial distribution with 20 possible deuteron positions and deuteron enrichment of 0.04, the (d-isotopomer distribution) generated for a peptide is $(0.4420, 0.3683, 0.1458, 0.0364, 0.0065, 0.0009)$. The isotopomers in the isotope envelop of the full peptide after deuteron incorporation is $(0.1462, 0.2845, 0.2733, 0.1729, 0.0810, 0.0300)$. The effects of deuteron enrichment on isotopomer distribution are shown in Fig. 4 using the concatenation function. The proper deuteron enrichment to be used in any experiment can be optimized using the concatenation operation. It can be shown that the inverse operation on the isotopomers in the isotope envelop of the full peptide and the prelabeling peptide (C-isotopomers) gives the (d-isotopomer distribution), which shows the effect of deuteron incorporation alone. Because of the low enrichment of deuterium used in animal studies, there is a good likelihood that some of the newly synthesized peptide does not have any deuteron incorporation. In such experiments, $m_0$ represents the sum of preexisting and newly synthesized peptide. However, the fractions represented by $m_1$ and $m_2$ are the results of deuteron incorporation. Thus, using the consecutive mass isotopomer equation, we can determine the $N(d)$ and $p(d)$, using the equation $m_2/m_1 = (N - 1)/2 \times p/d$, where $q = 1 - p$. After determining deuteron enrichment, we can estimate $N(d)$ and fraction of newly synthesized peptide by dividing the observed $m_1$ by the theoretical $m_1$. This is illustrated in Fig. 5.

Steps in the calculation of new fraction. We first estimated the deuteron enrichment in water to be 2.5% using deuteron incorporation into palmatine (data not shown) (Ref. 11). Using results of $m/z$ 1,393 in Tables 1 and 2, we set up the algorithm for the determination of deuteron isotopomers of the protein using the inverse concatenation operation. The regression matrices of the operation are shown below.

\[
\begin{bmatrix}
 0.4466 & 0.0000 & 0.0000 & 0.0000 & 0.0000 & \vdots \vdots \vdots \vdots \vdots \vdots \\
 0.3393 & 0.4466 & 0.0000 & 0.0000 & 0.0000 & \vdots \\
 0.1448 & 0.3393 & 0.4466 & 0.0000 & 0.0000 & \vdots \\
 0.0474 & 0.1448 & 0.3393 & 0.4466 & 0.0000 & \vdots \\
 0.0127 & 0.0474 & 0.1448 & 0.3393 & 0.4466 & \vdots \\
 0.0051 & 0.0127 & 0.0474 & 0.1448 & 0.3393 & \vdots \\
\end{bmatrix} \begin{bmatrix}
 b_0 \\
 b_1 \\
 b_2 \\
 b_3 \\
 b_4 \\
 \vdots \\
 b_n \\
\end{bmatrix} = \begin{bmatrix}
 0.2748 \\
 0.3392 \\
 0.2205 \\
 0.1025 \\
 0.0395 \\
 0.0160 \\
\end{bmatrix}
\]

Using a spreadsheet program (Excel), the output of such a multiple linear regression is shown below in Table 5, and the coefficients represent the distribution of mass isotopomers due to deuteron incorporation. Thus 61.5% of the molecules did not have any deuteron, 29.2% were labeled with one deuteron, and 7.3% were labeled with 2 deuteron atoms. Using the ratio of consecutive isotopomers relationships that $m_2/m_1 = [N(d) - 1]/2 \times p/d$, we determined the theoretical $N(d)$ to be 20. The theoretical deuteron isotopomers of $(20, 0.025)$ are $(0.6027, 0.3091, 0.0753, 0.0116, 0.0013)$ corresponding to fractions of molecules with $0, 1, 2, 3,$ and 4 deuteron substitutions. This is the theoretical distribution of deuteron isotopomers in the newly synthesized protein. It can be shown by multiple linear regression that observed distribution $(X_1, X_2, X_3, \ldots)$ accounts for 96.2% of the deuteron distribution in $m/z$ 1,393 or the new fraction is 0.9619.

The $m_2/m_1$ ratios were determined similarly for the other peptides of Table 3. The corresponding theoretical distributions were calculated. The new fraction of the peptide is the contribution of the theoretical distribution as a fraction of the observed d-isotopomer distribution.

ACKNOWLEDGMENTS

The rat samples analyzed in this study were obtained from Sherin U. Devaskar, who is supported by the National Institutes of Health (HD-41230), Department of Pediatrics at the David Geffen School of Medicine, after receiving approval from the UCLA Animal Research Committee.

Present address of G. G. Xiao: Dept. of Biomedical Sciences, Creighton Univ. Medical Center, 601 N. 30th St., Ste. 6730, Omaha, NE 68131.

GRANTS

This work was supported in part by National Institutes of Health Grant M01-RR-00425 of the General Clinical Research Unit, P01-AT-003960-01 UCLA Center for Excellence in Pancreatic Diseases, Metabolomics Core, and a grant from the Hirshberg Foundation for Pancreatic Cancer Research. The Voyager DE was purchased with a grant from the Henry Guenther Foundation.

REFERENCES


Downloaded from http://jap.physiology.org/ by 10.220.3.51 on June 20, 2017
Innovative Methodology

Determination of Protein Synthesis Using MIDA


