Bull. Chem. Soc. Ethiop. **2009**, 23(1), 101-103. ISSN 1011-3924
Printed in Ethiopia

SHORT COMMUNICATION

THE MASS OF CELLULAR RETINOIC ACID BINDING PROTEIN I INVESTIGATED BY ^{13}C DEPLETION AND MASS SPECTROMETRY

Lin Zhang[1*], Zeqing Song[2] and Yanmei Wen[1]

[1]College of Science, Guangdong Ocean University, Zhanjiang, 524088, China
[2]Department of Internal Medicine, Guangdong Medical College, Zhanjiang, 524001, China

(Received June 26, 2008; revised August 31, 2008)

ABSTRACT. The accuracy of mass spectrometry used to determine large molecular mass as proteins is often influenced by the isotopic compositions within a protein. Isotopic depletion is a powerful tool to resolve this problem. Using Fourier transform ion cyclotron resonance mass spectrometer, we investigated the ^{13}C depleted cellular retinoic acid binding protein I, the measured mass accuracy is increased compared with the natural protein.

KEY WORDS: Molecular mass, Mass accuracy, Isotopic depletion, Monoisotopic

INTRODUCTION

Determination of accurate molecular mass is a prerequisite qualification for protein characterization, and mass spectrometry is a powerful analytical technique used for the molecular mass analysis [1-3]. In general, the mass spectrum of molecular ion consists of a sum of signals from species of various possible isotopic compositions. Thus the mass spectrometry accuracy for detecting molecular mass can be limited by the broad isotopic envelope. This limitation is especially obvious for large molecules as proteins, because of the tendency of closely spaced peaks to coalesce into a single resonance, inducing difficult to resolve the protein isotopic fine structure. An effective strategy to overcome this issue was recently presented by Marshall *et al.* [4]. Compare to the use of natural-abundance isotopic distributions, depletion of rare isotopes can increase both sensitivity and molecular mass measurement accuracies, although the degree of isotopic depletion is often limited by cost. On the other hand, since the coalescence tendency scales inversely with magnetic field strength, one can hope to resolve isotopic fine structure at sufficiently high magnetic field strength. Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS) extends the ion cyclotron resonance principle to simultaneous detection of ions of multiple mass-to-charge ratios by pulsed excitation, and followed by Fourier transformation of the digitized response, producing ppb precision in atomic ion masses [5]. Here, we report the isotopic fine structure of pseudo-wild type cellular retinoic acid binding protein I (wt*-CRABP I) with a stabilizing mutation R131Q, a protein whose molecular mass up to 17 kDa utilizing ^{13}C depletion combined FT-ICR MS technology.

EXPERIMENTAL

The synthesis of ^{13}C depleted wt*-CRABP I was based on an adaptation of a previously published method [5] using *E. coli* and 99.9 % ^{12}C glucose. A 4.7T Apex II (Bruker Daltonics) Fourier transform ion cyclotron resonance mass spectrometer was employed to detect the effects of isotope depletion.

*Corresponding author. E-mail: zl_zhanglin@yahoo.com

RESULTS AND DISCUSSION

Although the natural abundances of rare isotopes are low (e.g. the abundances of ^{13}C and ^{15}N are 1.11 % and 0.36 %, respectively), a protein contains hundreds to thousands of such atoms as carbon and nitrogen, producing a wide distribution in the natural abundance of molecules. Figure 1 is the mass spectra of wt*-CRABP I (A) ^{13}C depleted protein and (B) natural isotopic protein utilizing FT-ICR MS. The mass spectrum of ^{13}C depleted protein is much narrower than that of natural abundance protein. Since the signal is "concentrated" in fewer peaks, the signal-to-noise ratio increases and leads to more accurate mass determination.

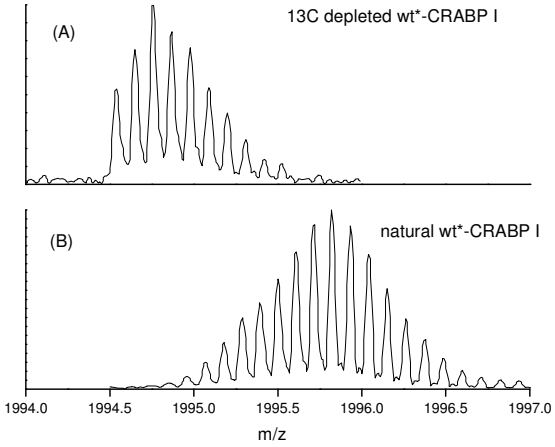

Figure 1. Mass spectra of the +9 charge state of (A) ^{13}C depleted wt*-CRABP I (B) natural wt*-CRABP I (20 μM protein in aqueous solution containing 50 % methanol and 3 % acetic acid).

In mass spectrum of ^{13}C depleted protein and natural protein, the only peak that has a unique elemental composition is the monoisotopic peak, which has the narrowest mass distribution thus potentially highest mass accuracy for most biological macromolecules. Whereas the monoisotopic peak of mass spectrometry may be below the noise level and higher isotopes may dominate completely. For most biological macromolecules with molecular weight greater than 10 kDa, the monoisotopic relative abundance is so low that almost undetectable [6], because the tiny peak is buried in the background noise in the spectrum. For isotopic depleted biomolecules, the monoisotopic peak is much higher in abundance and easily recognized by its sudden onset. Figure 2 shows the theoretical mass spectra of wt*-CRABP I with depleted (A) and natural (B) isotopic distribution. The monoisotopic mass of wt*-CRABP I is 17941.78 Da, corresponding to mass-to-charge ratio (m/z) 1994.54 for +9 charge state. The calculated monoisotopic abundance with Isopro software is 0.0025 % for natural protein, whereas the monoisotopic abundance is 9.99 % for ^{13}C depleted protein (22.78 % for $^{13}C^{15}N$ doubly depleted protein). As shown in Figure 1(A), the monoisotopic peak abundance is now high enough to being recognized by its sudden onset, in good agreement with the theoretical result.

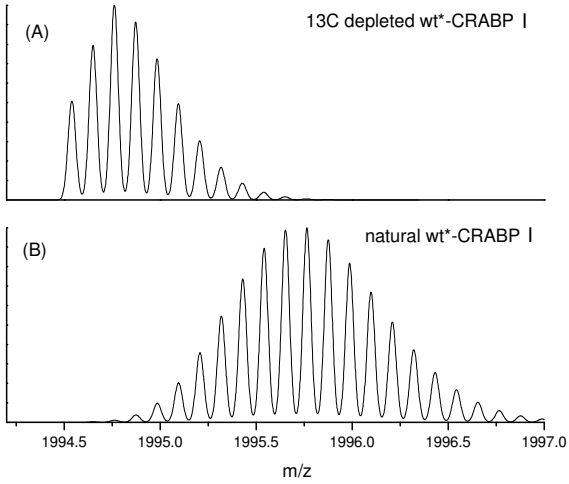

Figure 2. Theoretical mass spectra of the +9 charge state of ^{13}C depleted wt*-CRABP I (B) natural wt*-CRABP I.

As discussed above, compared to the mass spectra of natural protein, ^{13}C depleted protein greatly enhanced abundance of monoisotopic ions (by a factor of nearly 73) and a narrower isotopic distribution with higher signal-to-noise ratio, so that there is no ambiguity about assigning the correct molecular weight to the nearest Dalton. Our result indicates that for large molecules as proteins, depletion of rare isotopes combining mass spectrometry with high magnetic field strength can provide an effective strategy to increase both sensitivity and molecular mass measurement accuracies

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