Isotopes and mass spectrometry - dawn of history to today

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Early History of Mass Spec

• 1897 Rutherford discovers the electron (cathode rays)

• 1919 Aston using a mass spectrograph shows that Neon with a non-integer MW (20.2 Da) is composed of two isotopes, $^{20}\text{Ne}$ and $^{22}\text{Ne}$
Mass spectrometry and nuclear war

- 1935 Dempster discovers $^{235}$U, the uranium isotope first used for a nuclear fission (atom) bomb
- 1941-5 Manhattan project
  - Mass spectrometry, one of three methods used to isolate $^{235}$U
- Bainbridge’s careful measurement of masses of the elements revealed that hydrogen was heavier than predicted
  - This led to the concept that fusion of four H atoms to form He would result in the loss of mass in the form of energy - from Einstein, $E = mc^2$, i.e., a whole bunch!
    - Predicted mass for helium = 4.03298 vs actual of 4.02602
    - $\Delta = 0.00695$

Isotope profile of an individual peptide ion

1 Da apart. Is the ratio constant?
Elements

Carbon-12  
6 protons  
6 neutrons  
6 electrons  
Stable

Carbon-13  
6 protons  
7 neutrons  
6 electrons  
Stable

Carbon-14  
6 protons  
8 neutrons  
6 electrons  
Unstable  
Neutron decays to produce a proton, a β-particle and an anti-neutrino - \(^{14}\)C becomes \(^{14}\)N

Stable isotopes of the most abundant elements found in biological materials

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*Varies according to its source
Formation of helium

The first step involves the fusion of two hydrogen nuclei $^1\text{H}$ (protons) into deuterium, releasing a positron and a neutrino as one proton changes into a neutron.

$$^1\text{H} + ^1\text{H} \rightarrow ^2\text{D} + e^+ + \nu_e + 0.42 \text{ MeV}$$

This first step is extremely slow, both because the protons have to tunnel through the Coulomb barrier and because it depends on weak interactions.

The positron immediately annihilates with an electron, and their mass energy is carried off by two gamma ray photons.

$$e^- + e^+ \rightarrow 2\gamma + 1.02 \text{ MeV}$$

After this, the deuterium produced in the first stage can fuse with another hydrogen to produce a light isotope of helium, $^3\text{He}$:

$$^2\text{D} + ^1\text{H} \rightarrow ^3\text{He} + \gamma + 5.49 \text{ MeV}$$

From here there are three possible paths to generate helium isotope $^4\text{He}$. In pp I helium-4 comes from fusing two of the helium-3 nuclei produced; the pp II and pp III branches fuse $^3\text{He}$ with a pre-existing $^4\text{He}$ to make Be. In the Sun, branch pp I takes place with a frequency of 86%, pp II with 14% and pp III with 0.11%. There is also an extremely rare pp IV branch.

http://en.wikipedia.org/wiki/Nuclear_fusion

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The $^{12}\text{C}/^{13}\text{C}$ ratio

- $^{12}\text{C}$ was born in the inferno of stars from the triple fusion of $^4\text{He}$ nuclei
- $^{13}\text{C}$ is present in varying ratios to $^{12}\text{C}$ among different stars and galaxies
- Carbon is present in interstellar space as CN and CO, and as methane and other hydrocarbons in planets
  - Thaolins are in the atmosphere of the moon Titan - future source of life?
The CNO cycle - Bethe-Weizsäcker-cycle

The CNO cycle starts occurring at ~ 13×10^6 K, but its energy output rises much faster with increasing temperatures. At ~ 17×10^6 K, the CNO cycle starts becoming the dominant source of energy. The sun has a temperature of around ~ 15.7×10^6 K and only 1.7% of ^4He nuclei being produced in the Sun are born in the CNO cycle.

\[
\begin{align*}
^{12}\text{C} + ^1\text{H} &\rightarrow ^{13}\text{N} + \gamma + 1.95 \text{ MeV} \\
^{13}\text{N} &\rightarrow ^{12}\text{C} + ^1\text{H} + ^1\text{H} + ^1\text{H} + ^2\text{He} + 2.22 \text{ MeV} \\
^{13}\text{C} + ^1\text{H} &\rightarrow ^{14}\text{N} + \gamma + 7.54 \text{ MeV} \\
^{14}\text{N} + ^1\text{H} &\rightarrow ^{15}\text{O} + \gamma + 7.35 \text{ MeV} \\
^{15}\text{O} &\rightarrow ^{12}\text{C} + ^3\text{He} + 2.75 \text{ MeV} \\
^{15}\text{N} + ^1\text{H} &\rightarrow ^{12}\text{C} + ^3\text{He} + 4.96 \text{ MeV}
\end{align*}
\]

http://en.wikipedia.org/wiki/CNO_cycle

Terrestrial ^{12}\text{C}/^{13}\text{C} ratio

- On average there is 1.11% of ^{13}\text{C} amongst the total carbon on Earth
- Carbon is present in many forms accessible to synthetic and biosynthetic processes - mostly starting from CO\textsubscript{2}
- CO\textsubscript{2} is in the atmosphere, in the sea as HCO\textsubscript{3}⁻, in the soil as carbonates, and as organic intermediates
  - ^{12}\text{C} and ^{13}\text{C} partition differently in each of these environments due to physical effects
  - Compounds have different ^{12}\text{C}/^{13}\text{C} ratios
Isotope ratio mass spectrometry

- Used for $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$, $^{18}\text{O}/^{16}\text{O}$
- Carbon compounds are converted to CO$_2$
- Low mass range 0-150 $m/z$
- Sector instrument
- Very sensitive
- Very accurate measurement of mass
- $^{13}\text{C}/^{12}\text{C}$ ratio can vary from 0.972% to 1.160%
- PeeDee standard has $^{13}\text{C}/^{12}\text{C}$ ratio of 1.12372%
Fixation of CO$_2$ as organic carbon

- RuBisCO - enzyme complex in plants
  - Converts CO$_2$ to sugars
  - Prefers $^{12}$C to $^{13}$C
- Plants take in CO$_2$ through stomata
  - Two models
    - Sponge divers (intermittent breathing)
      - These would sample all the isotopic forms of CO$_2$
    - Swimmers on surface (frequent breathing)
      - These would selectively take in $^{12}$CO$_2$

$^{12}$C/$^{13}$C ratio in plants

- The $^{13}$C content would be higher in plants that held their breath like the divers
  - i.e., the stomata were open less frequently
- Drought-resistant wheat strains have a higher $^{13}$C/$^{12}$C ratio
  - This is a marker for selection of drought-resistant strains, important in the coming global warming
Athletes who use synthetic testosterone

- Synthetic testosterone is made from phytosterol perursors, typically derived from wild yams or soy.
- Those are both warm-climate C3 plants, which take up atmospheric carbon dioxide by a different route than temperate-zone C4 plants, leading to noticeably different isotope ratios.
- The typical Western industrial-country diet is derived from a mixture of C3 and C4 stocks, so the appearance of testosterone with a C3-plant isotopic profile is usually diagnostic.

The ultimate mass spectrometer

Accelerator mass spectrometer
Set up of AMS at Purdue

10 GeV AMS at LLNL
Ions (C−) are generated by a Cs ion beam. The 12C− ions are removed by a low energy mass spectrometer (note 14N does not form ions). The remaining ions are accelerated and on passage through the gold foil stripper form C4+ ions. The ions are separated based on momentum, thereby measuring the 13C+ ions. The ions are further selected for their m/z values in the Rigidity filter, and velocity in the Wien filter. They are finally measured using a multi-anode gas-ionization detector. Approximately 1% of the 14C ions generated are detected. Sensitivity is ~10 attomoles of 14C from mg sized samples.

**Sensitivity of 14C-AMS**

- If one carbon atom is incorporated into a compound, then the specific activity is ~50 μCi/μmol
- The human body naturally contains 50-90 nCi of radioactivity – therefore, a 50 nCi, dose is reasonable and small compared to most clinical studies
- 50 nCi is 1 nmol (10⁻⁹ mole)
- AMS can measure 1 14C atom in 10¹⁵ carbon atoms, or 10 attomoles (10⁻¹⁷ moles) – 10⁻⁸ of the dose
- For a 70 kg human, 1 mg of tissue represents 1.4 x 10⁻⁵.
- If distributed evenly, the S/N is 700:1
14C-polyphenols by metabolic labeling

Plant cells incubated with 14C-labeled sucrose in a closed system

14C-labeled polyphenols extracted and fractionated

50 nCi of 14C-labeled polyphenol(s) is $1.11 \times 10^5$ dpm or $1 \times 10^{-9}$ mol (0.3 µg)

Let's suppose that 0.1% of the dose is absorbed into the brain, then that is 111 dpm, 0.3 ng or $1 \times 10^{-12}$ mol

For a 3 g rat brain, then 3 mg is 0.11 dpm, 0.3 pg or $1 \times 10^{-15}$ mol

The detection limit for 14C using AMS is $1 \times 10^{-17}$ mol, i.e., S/N is 100:1

Differential retention of 14C-labeled proanthocyanidin by AMS

Elsa Janle et al.
Can isotope ratios be ascertained for compounds?

- Conventional isotope ratio measurements are based on converting the carbon-containing compounds to CO2.

- Given the improvements in mass spectrometry, could we investigate the isotope ratios of peptides and deduce the underlying $^{13}$C/$^{12}$C ratio?

Fragmentation of a peptide
Expected isotope abundances

- $x$ is the fraction of carbon atoms that are $^{12}\text{C}$
- $y$ is the fraction of carbon atoms that are $^{13}\text{C}$
- For 1 carbon, the distribution is $x:y$
- For 2 carbons, $x^2:2xy:y^2$
- Using the binomial expansion
  - For $n$ carbons, $x^n; nx^{(n-1)}y; (2n-1)x^{(n-2)}y^2; \ldots$
  - $x^n$ are all $^{12}\text{C}$; for the next isotope peak there is one $^{13}\text{C}$
  - The ratio ($r$) of those first two peaks = $ny/x$
  - But $x+y=1$, so $x=1-y$, hence $r = ny/(1-y)$ and $r-ry=ny$
  - Further, $y(n+r)=r$, and therefore $y = r/(n+r)$

Calculating the $^{13}\text{C}/^{12}\text{C}$ ratio in keratin peptides

- Observe peptides with clean isotope profiles
- Identify the peptide from their MS/MS spectra
  - Determine the number ($n$) of carbon atoms in the peptide
- Calculate the areas under the observable isotope peaks
- Estimate the $^{13}\text{C}/^{12}\text{C}$ ratio using the correction for $n$
Partial list of proteins in hair soluble fraction by LC-tandem MS

- Keratin 3A [ Homo sapiens ]
- Keratin 3B [ Homo sapiens ]
- Keratin 3I [ Homo sapiens ]
- Hair keratin 3II [ Homo sapiens ]
- Keratin, hair, acidic, 4 [ Homo sapiens ]
- Keratin 34 [ Homo sapiens ]
- Hair type II basic keratin [ Homo sapiens ]
- Keratin 05 [ Homo sapiens ]
- Type II hair keratin 3 [ Homo sapiens ]
- Chain B, Non-Covalent Complex Between Alpha-1-Pi-Pittsburgh And 8895a Trypsin
- Keratin 12 [ Homo sapiens ]
- Keratin 12 [ Homo sapiens ]
- Keratin 12 [ Homo sapiens ]
- Keratin 10 [ Homo sapiens ]
- Keratin 10 [ Homo sapiens ]
- Keratin associated protein 3.1 [ Homo sapiens ]
- Keratin 1 [ Homo sapiens ]
- Hair keratin 3I [ Homo sapiens ]
- Hair keratin 3II [ Homo sapiens ]
- Keratin 13 (isoform s) [ Homo sapiens ]
- Vimentin [ Homo sapiens ]
- Vimentin [ Homo sapiens ]
- Vimentin [ Homo sapiens ]
- Vimentin [ Homo sapiens ]
- Collagen-like 1, isoform A [ Homo sapiens ]
- Collagen-like 1, isoform A [ Homo sapiens ]
Tandem mass spectrum of DSLENTLTESEAR, 733.0 m/z

Elemental composition - $C_{58}H_{98}N_{17}O_{27}$
Chromatograph of 732.7 to 733.2 amu with 30.92 min representing the 732.9 m/z peak.

EMS Scan of 733 m/z (2′) with resulting Enhanced Resolution scan of 732.9 m/z

All-^{12}C → 1-^{13}C peak
Estimating $^{13}$C content

- The ratio of the $^{1-13}$C and $^{12}$C isotope peak heights is $2.18/2.28$, i.e., $r = 0.95614$
- $Y$, the fraction of the carbons that are $^{13}$C, is $r/(n+r)$
- $Y = 0.95614/(58 + 0.95614)$
- $Y = 0.015947$, i.e. 1.59%

Mass differences for H, C, N and O isotopes

- $^2$H - $^1$H = 1.0063 Da
- $^{13}$C - $^{12}$C = 1.0031 Da
- $^{15}$N - $^{14}$N = 0.9970 Da
- $^{17}$O - $^{16}$O = 1.0042 Da
- Therefore, $^{13}$C and $^{15}$N will be different by -0.0061 Da and $^{13}$C and $^{17}$O by 0.0011 Da
- The $^2$H and $^{13}$C difference would be 0.0032 Da, but only accounts for 1% of the apparent $^{13}$C difference
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\[ ^{15}\text{N}/^{14}\text{N} \] ratio over the archaea periods

- Earth did not start with an oxygen atmosphere
  - Dominated by a \( \text{N}_2/\text{NH}_3/\text{CN}^- \) anerobic environment for first several billion years
  - First settlers on Earth were archaea bacteria
  - A nitrogen-fixing archaeon from a deep sea volcanic vent operates at 92°C
Processes leading to changing $^{15}$N/$^{14}$N ratio

Examination of the $^{15}$N/$^{14}$N ratio in 2.5 billion year old Mount McRae Shale in W. Australia reveals a transient period of nitrification and implies that nitrifying and denitrifying bacteria were already present.

Garvin et al., Science 323, 1045 (2009)

$^{18}$O/$^{16}$O isotope ratio

- Evaporation of H$_2$$^{18}$O requires more energy than H$_2$$^{16}$O
  - Water vapor is enriched in $^{16}$O whereas ice is enriched in $^{18}$O
  - Measurement of $^{18}$O/$^{16}$O ratio in ice cores allows scientists to estimate the temperature over the past millions of years
  - As the temperature decreases, the $^{18}$O/$^{16}$O ratio falls
  - Hurricanes cause a severe depletion of $^{18}$O – this can be detected in trees
  - Calcite (shells) takes one O from water and parallels the ice record
The association of $\delta^{18}O$ changes with tropical storms.

The bulk time-dependent change in $^{18}O$ is modeled and then fitted to the isotope record as measured in tree rings.

The known tropical cyclones are associated with significant deviation to lower amounts of $^{18}O$.

Miller et al., PNAS 103:14294, 2006
$^{15}\text{N}$ and the $^{13}\text{C}_1$ isotope abundance

- It appears that there is a contribution from the $^{15}\text{N}$ and $^{17}\text{O}$ isotopes
  - For $^{15}\text{N}$ it is estimated as $(0.36/1.11)/(58/17) = 0.09506$ in $r$
  - Thus $r^* = 0.86108$ and $y^* = 0.86106/(58 + 0.86104)$, i.e., $0.014629$, or $1.4629\%$

Conclusions so far

- The method using comparison of the isotope peaks of a peptide is not sufficiently good (yet)
- The calculations used in this first approach employed peak heights
  - It would be better if we could deconvolute the overlapping peaks to obtain estimate of the peak areas
  - Better still to resolve the peaks entirely and separate the isotopic contaminants
Mass resolution on different instruments

Can high resolution FT-ICR-MS resolve the $^{13}\text{C}$, $^{15}\text{N}$ isotope contributions?

- $\Delta^{13}\text{C} - ^{12}\text{C} = 1.0034$ Da
- $\Delta^{15}\text{N} - ^{14}\text{N} = 0.9970$ Da
- Therefore, the difference between a $^{13}\text{C}$ and $^{15}\text{N}$ contribution is 0.0064 Da
- The $m/z$ of the doubly charged keratin peptide is 733
- Resolution needed is $2 \times 733/0.0064 = 229,063$
- Achievable on 7T FT-ICR instrument if the FID is observed for several seconds