MASS SPECTROMETRIC DETERMINATIONS OF TRYPTOPHAN AND ITS METABOLITES

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INTRODUCTION

The unique character of a chemical compound is determined by its molecular architecture. That architecture is a sum not only of all of the atoms in the compound and their masses, but of their particular relationship in space, i.e., the molecular bonds, sub-structural components, and their stereochemistry. Consequently, a physical tool which can measure both the summed masses of the elements in a compound and reflect the subtleties of their arrangement is very useful in both quantitative and qualitative investigations in biochemistry.

Mass spectrometers can measure the mass of small quantities of most organic compounds accurately, and because of the techniques associated with the mass measurement process, these instruments generally reflect very subtle differences in molecular structure. There is now a large variety of mass spectrometers employed in biochemistry, but there are several principles which they share. First, a neutral organic molecule cannot be directed in space, but once ionized, it can be accelerated, steered, focussed, or otherwise directed by either electric or magnetic fields. Second, an ionized molecule can only be directed by external fields if its movement is unrestricted by the action of other molecules. Consequently, all mass spectrometers require high vacuum in the region of the mass analyzer. In fact, most of the cost and physical size of mass spectrometers is determined by this requirement. Third, the quantity of ionized compound required for modern detectors is in the range of hundreds or thousands of ions (10^{-21} or 10^{-20} moles), so that the instrumentation is inherently sensitive. However, due to the low efficiency of most ionization processes, current detection limits are 10^{-15} to 10^{-18} moles, and those limits are for the most favorable cases. Nevertheless, mass spectrometry remains among the most sensitive and specific of the physical chemical measurement methods.

What these general characteristics mean to biochemists studying tryptophan metabolism is that mass spectrometry is an appropriate tool for high sensitivity measurements requiring a high degree of structural differentiation, but that there will be many alternative specific approaches to any
measurement to be made. Two major components of compound differentiation available to the mass spectroscopist are the choice of how a compound is to be presented to the instrument (gas, solid, liquid, chromatographic effluent, etc.) and the method of its subsequent ionization (electron impact, fast atom bombardment, thermospray, etc.). In most of the examples which follow, online gas chromatography has been the method of choice for sample introduction into the mass spectrometer. This is because most biological fluids and their extracts contain very complex mixtures of materials. By coupling a separation technique with mass analysis, an additional element of selectivity is introduced. Further, online and automated gas or liquid chromatography provides a convenient way to admit samples sequentially to analytical instrumentation. A chromatographic column can absorb most of the chemical insult which would otherwise be directed into an ionization chamber. Interposing a removable and disposable guard element between the analyst and the instrumentation minimizes maintenance and maximizes usable instrument time. The result of improvements in all of the elements of chromatographic-mass spectrometric systems is that this instrumentation is highly reliable, faster, less expensive, easier to use, and consequently, a preferred analytical method for many applications. Gas chromatography requires that the compounds of interest be stable in the vapor phase, a requirement not met by tryptophan metabolites unless chemically derivatized for that purpose. Much of the analytical literature describing measurements of metabolites by gas chromatography-mass spectrometry (GC/MS) is largely devoted to derivatization chemistry.

While sensitivity and selectivity are attainable with mass spectrometric instrumentation, there are many other sensitive and selective detectors for gas and liquid chromatography, as well as highly sensitive immunoassay methods. Many of these alternatives are both less costly and simpler to use. However, there is another property of organic compounds which defines the utility of mass spectrometry in biochemistry, and that is the occurrence of stable isotopes of carbon (\(^{13}\)C), nitrogen (\(^{15}\)N), oxygen (\(^{18}\)O), and hydrogen (\(^{2}\)H or D). Physically and chemically, all of the isotopes of an element are nearly indistinguishable. In nature, the low abundance of stable, non-radioactive, isotopes of carbon, nitrogen, oxygen, and hydrogen makes their relative contribution to a complex molecule small. That is, while \(^{13}\)C, the most common of these, occurs at an abundance of 1.1% of \(^{12}\)C, the chance of having more than one \(^{13}\)C in any given molecule is \(n(1.1\%)^y\), where \(n\) is the number of carbon atoms in a molecule, and \(y\) equals the number of multiple \(^{13}\)C atoms. Consequently, molecules intentionally synthesized containing a high abundance of one or more stable isotopes will be unique in nature, while retaining the chemical properties of the natural material. Stable isotope labeled variants of compounds are known as "isotopomers". Isotopomers will generally be indistinguishable from the natural material with regard to chemical reactivity and physical properties, with the exception of molecules highly enriched with \(^{2}\)H. That is, the mass of the rarer isotopes of carbon, nitrogen, and oxygen differs by approximately ten percent from each of the respective more abundant isotopes, whereas the hydrogen-deuterium mass difference (1 vs. 2 daltons, or 100%) confers significant differences in bond strength and polarity. Only mass selective detection will differentiate most isotopomers, and thus they are ideal species for tracing metabolic pathways, measuring kinetics, or as internal standards for quantitative analysis.

After reviewing the tryptophan literature, we have chosen several examples of qualitative and quantitative measurements which illustrate mass spectrometry as a tool in tryptophan metabolism studies.

QUALITATIVE APPLICATIONS OF MASS SPECTROMETRY

Characterization of an unknown metabolite: The unambiguous demonstra-