Strategies for Probing Metabolism

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1. Introduction

The proper functioning of living systems, ranging from microbes to humans, rests on the interplay between gene expression and transcription, protein expression and metabolite dynamics. The interconversion of metabolites and the regulation of metabolite levels by proteins are often referred to as metabolism and NMR spectroscopy is a unique technique to quantitatively study many important aspects of metabolism non-invasively *in vivo*. Direct ^{1}H NMR spectroscopy allows the detection of a wide range of metabolites in the milli-to-micromolar concentration range, including components of energy metabolism, neurotransmitters and lipids. However, static metabolite concentrations only represent one part of metabolism. The dynamic synthesis and degradation of metabolites over time underlies their apparent static levels and a complete understanding of metabolism thus also requires knowledge of these dynamic processes. Fortunately, NMR spectroscopy provides several different strategies to probe the dynamic aspects of metabolism at various time scales.

2. ^{31}P NMR spectroscopy

Phosphorus-31 NMR signals were among the first observed *in vivo* because of the 100% natural abundance, spin-1/2 properties, relatively high sensitivity and relative ease of acquisition (e.g. water suppression is not required). ^{31}P NMR spectra are often relatively simple with resonances from phosphocreatine (PCr), adenosine triphosphate (ATP), inorganic phosphate (P_i) and phosphomono- and diesters. On several time scales the ^{31}P NMR resonances are very dynamic. For example, the inorganic phosphate resonance is in rapid exchange between single- and double-protonated forms (around pH = 7). As the exchange is very fast relative the frequency difference between the two P_i resonances, only a single P_i resonance will be observed of which the chemical shift is dependent on the pH (1). Similarly, the chemical shift of the ATP resonances is dependent on the pH and magnesium concentration. These exchange reactions are typically very fast because they are not catalyzed by enzymes. Many of the compounds observed by ^{31}P NMR are involved in very important, enzyme-catalyzed reactions, such as ATP synthesis and the interconversion of PCr and ATP by creatine kinase. Magnetization transfer methods (2,3) can be used in combination with ^{31}P NMR to quantitatively study the fluxes through these metabolic pathways (4-6). Magnetization transfer methods are limited to exchange reactions that are fast relative to the longitudinal T_1 relaxation rate. Much slower reactions can be probed with ^{13}C and ^{17}O NMR spectroscopy.

3. ^{13}C NMR spectroscopy

Almost every biologically important compound contains carbon thereby making ^{13}C NMR spectroscopy, in principle, a very important technique to detect a wide range of metabolites.

However, the common carbon isotope, ^{12}C, is not NMR active whereas the NMR active isotope, ^{13}C is only present at a 1.1% natural abundance. This, in combination with the fact that the NMR sensitivity of ^{13}C NMR is relatively low when compared to ^{1}H NMR, has prevented ^{13}C NMR from becoming a routine NMR detection method. When combined with the intravenous infusion of ^{13}C-enriched substrates, like [1-^{13}C]-glucose, ^{13}C NMR spectroscopy is uniquely capable of following the flow of the ^{13}C label from the substrate into a wide variety of products, including glutamate, glutamine, γ-aminobutyric acid (GABA) and glycogen. This provides quantitative information about the metabolic fluxes through the tricarboxylic acid (TCA) cycle, excitatory and inhibitory neurotransmission and glycogen synthesis (7-13).

4. ^{17}O NMR spectroscopy

While ^{13}C NMR provides a wealth of information on a large number of metabolic pathways, it is a technically challenging method that is still not routinely available in a clinical setting. ^{17}O NMR can provide an alternative technique to probe one specific metabolic rate, namely oxygen consumption, faster and perhaps easier (14-17). Similar to ^{13}C NMR, ^{17}O NMR detection requires isotope-enriched substrates due to the low natural abundance of only 0.037% (18). ^{17}O is a spin-5/2 nucleus with very short T_1 and T_2 relaxation times which can be used to greatly enhance the sensitivity by extended signal averaging (19). During oxidative metabolism, molecular oxygen is converted to water at a rate that is closely related to other energy producing pathways, like the TCA cycle. This conversion can be detected with ^{17}O NMR spectroscopy during the inhalation of ^{17}O-enriched oxygen gas. The relatively high sensitivity of ^{17}O NMR allows the generation of metabolic maps depicting the spatial dependence of oxygen consumption rates.

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