Direct Monitoring by Carbon-13 Nuclear Magnetic Resonance Spectroscopy of the Metabolism and Metabolic Rate of $^{13}$C-Labeled Compounds in Vivo

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Carbon-13 nuclear magnetic resonance spectroscopy has been used to observe the transformations of $[1-^{13}$C$]$-d-glucose to $[1,1'-^{13}$C$]$-d-trehalose, and $[3-^{13}$C$]$-l-alanine to $[2-^{13}$C$]$-l-glutamic acid in the living body of Gryllodes sigillatus. $[3-^{13}$C$]$-d-Alanine was not metabolized.

The metabolic rate of $[1-^{13}$C$]$-d-glucose was found to be altered by prior injection of boric acid.

Keywords $^{13}$C-NMR in vivo; $^{13}$C-glucose; $^{13}$C-trehalose; $^{13}$C-alanine; $^{13}$C-glutamic acid; metabolism; metabolic rate; boric acid

Introduction

The stable isotope carbon-13 ($^{13}$C), which is in nature at 1.1%, is useful in biochemical research because labelled positions can be easily identified by carbon-13 nuclear magnetic resonance spectroscopy ($^{13}$C-NMR) of extensive chemical shift without chemical degradation. It is very important in a wide area that the metabolic pathways in living body is directly investigated. Therefore we are interested in developing this method to monitor in vivo systems. The use of superconducting magnets provides extremely stable magnetic fields which can be adjusted with high sensitivity, so that it is possible to measure $^{13}$C-NMR signals without a deuterium lock system and without spinning the nuclear magnetic resonance (NMR) tube. The pulse sequence of NMR is also a matter of great importance to avoid tissue damage to living organisms.

Glucose is an energy source, and trehalose, which is produced from glucose, is an important blood sugar of insects.$^3$ Further, alanine is metabolized to glutamic acid, a neurotransmitter. Therefore, $[1-^{13}$C$]$-d-glucose (1), $[3-^{13}$C$]$-l-alanine (3), and $[3-^{13}$C$]$-d-alanine (4) were each injected into Gryllodes (G.) sigillatus, and their transformation in all a living body was directly monitored by $^{13}$C-NMR. The effect of boric acid on the metabolic rate of $[1-^{13}$C$]$-d-glucose (1) was also examined.

Results and Discussion

$[1-^{13}$C$]$-d-Glucose (1) was intraabdominally injected into G. sigillatus. After 30 min, the C-1 signals of injected $[1-^{13}$C$]$-d-glucose (1) were observed at 92.8 ppm (α-form) and 96.7 ppm (β-form) by $^{13}$C-NMR in vivo.$^4$ After 1 h, a new signal due to $[1,1'-^{13}$C$_2]$-d-trehalose (2) appeared at 93.9 ppm$^4$ and increased there after in parallel with the decrease of the signals at 92.8 and 96.7 ppm. After 4 h, $[1-^{13}$C$]$-d-glucose (1) had been completely metabolized in the body of G. sigillatus (Fig. 1). Thus, $[1-^{13}$C$]$-d-glucose (1) was not subjected to glycolysis, but was predominantly metabolized to $[1,1'-^{13}$C$_2]$-d-trehalose (2) (Fig. 2). The metabolite was isolated from G. sigillatus in 27% yield, and identified as $[1,1'-^{13}$C$_2]$-d-trehalose (2) by $^{13}$C-NMR, and fast atom bombardment mass spectrometry (FAB-MS).$^5$

Boric acid, which is said to be toxic to the digestive

![Fig. 1. $^{13}$C-NMR Spectra of the in Vivo Transformation of $[1-^{13}$C$]$-d-Glucose (1) in G. sigillatus (a) and Expanded $^{13}$C-NMR Spectra from 80 to 110 ppm (b)](image-url)

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processes and to decrease metabolism, usually used as an antihelmintic for the cockroach. We investigated boric acid have effects upon the metabolism of D-glucose. When [1,13C]-D-glucose (1) was intraabdominally injected into G. sigillatus 30 min after boric acid, the transformation of [1,13C]-D-glucose (1) to [1,1,13C2]-D-trehalose (2) was completed within 1 h (Fig. 3). Thus the metabolism of [1,13C]-D-glucose (1) to [1,1,13C2]-D-trehalose (2) unexpectedly was speeded up by boric acid.

Next, [3,13C]-L-alanine (3) was intraabdominally injected into G. sigillatus. After 1 h, the C-3 signal of injected [3,13C]-L-alanine (3) was observed at 17.9 ppm by 13C-NMR in vivo.35-36 After 2.5 h, a new signal appeared at 55.8 ppm, and this was assignable to C-2 of L-glutamic acid (5) formed via D-trehalose. The signal of [3,13C]-L-alanine (3) finally disappeared. However, when [3,13C]-D-alanine (4) was injected into G. sigillatus, it was not transformed.
Fig. 4. $^{13}$C-NMR Spectra Showing the in Vivo Transformation of $[3^{-13}$C]-l-Alanine (3) in G. sigillatus (a) and Expanded $^{13}$C-NMR Spectrum at 4 h (b)

(Fig. 5). $[3^{-13}$C]-l-Alanine (3) was presumably metabolized to $[3^{-13}$C]pyruvic acid (6). $[3^{-13}$C]Pyruvic acid (6) would then be converted into $[3^{-13}$C]oxaloacetic acid (7) in the TCA cycle, and successively to pro-R methylene $^{13}$C-labeled citric acid (8), $[4^{-13}$C]-α-ketoglutaric acid (9), and $[2^{-13}$C]-l-glutamic acid (5). The $[3^{-13}$C]pyruvic acid (6) was not
significantly metabolized to [2-13C]acetyl-Coenzyme A (10), which would lead successively to pro-S methylene
13C-labeled citric acid, [2-13C]-α-ketoglutaric acid, and
finally [4-13C]-L-glutamic acid (Fig. 2).

Experimental

**Instruments** 13C-NMR spectra in vivo were recorded 100 MHz on a
JEOL GSX-400 spectrometer with 10 mm multinuclear probes, referenced to
CDCl3 as an external standard. The spectral width was 24 kHz with
32 k data points, which corresponds to a resolution of 1.47 Hz per point.
The determined 90° pulse width was 8.0 μs, the acquisition time was
0.021 s, the pulse delay time was 1.0 s, and the probe temperature was
27°C. The pulse sequence of proton irradiation involved gate decoupling
without nuclear Overhauser effect (NOE). The spectra of samples were
measured at an organism without deuterium lock and without samples
spinning.

**Materials** The insects used were adult male G. sigillatus. [1-13C]-α-
Glucose (99 atom% 13C) was supplied by Cambridge Isotope Laboratories.
were prepared from 13C-iodomethane (99 atom% 13C), which was ob-
tained from Cambridge Isotope Laboratories.

**Measurement of Metabolism of [1-13C]-α-Glucose, and [3-13C]-α- and
L-Alanine by NMR in Vivo** A solution of [1-13C]-α-glucose, [3-13C]-α-
alanine or [3-13C]-L-alanine (2.0 mg) in water (20 μl) was intraabdomi-
nally injected into an insect, which was put into an NMR tube, and the
time course of signals was observed by 13C-NMR.

**Measurement of Metabolism of [1-13C]-α-Glucose in the Presence
of Boric Acid by NMR in Vivo** A solution of boric acid (1.29 mg) in water
(10 μl) was intraabdominally injected into G. sigillatus. After 30 min, a
solution of [1-13C]-α-glucose (2.0 mg) in water (10 μl) was intraabdomi-
nally injected, and the insect was put into an NMR tube. The time course
of signals was observed by 13C-NMR as above.

**Conclusion** The transformation of 13C-labeled compounds was suc-
cessfully monitored by in vivo 13C-NMR. The effect of an
added drug on the metabolic rate was also observed to
determine as the time proceeds. And the difference of metabolism between α-amino acid and γ-amino acid was
first observed, the metabolic pathways were confirmed for
13C-labeled position of metabolite. This technique could be
applicable to the studies of the metabolism of various drugs
and agricultural chemicals.

Acknowledgement We wish to thank Mr. Kazuhiro Matusita of JEOL
Co. and Dr. Katsuyuki Kurumaya for many helpful suggestions during
the course of this work.

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