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

Katsumi IIDA,[a] Osamu HIDOH,[b] Junichi FUKAMI,[b] and Masahiro KAJIWARA*,[a]

Department of Medicinal Chemistry, Meiji College of Pharmacy, 1–22–1 Yato-cho, Tanashi-shi, Tokyo 188, Japan[a] and 1–35–23 Nozawa, Setagaya-ku, Tokyo 154, Japan.[b] *Received July 2, 1990*

Carbon-13 nuclear magnetic resonance spectroscopy has been used to observe the transformations of [1-^{13}C]-D-glucose to [1,1′-$^{13}C_2$]-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[1] because labeled 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.[2] 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*.[3] 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

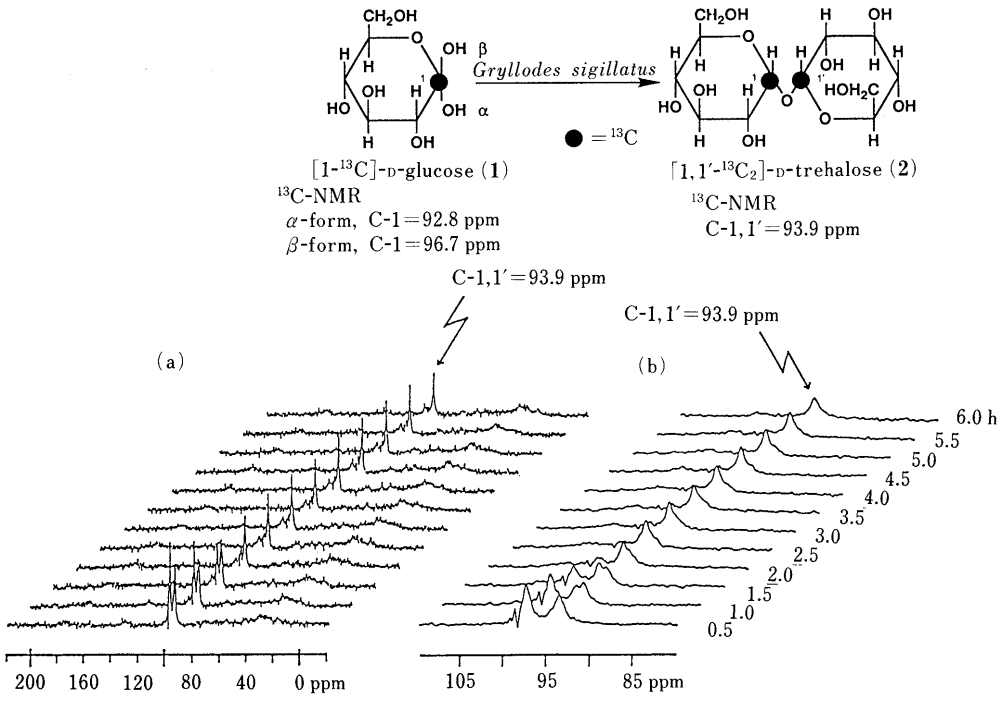

Fig. 1. ^{13}C-NMR Spectra of the *in Vivo* Transformation of [1-^{13}C]-D-Glucose (**1**) in *G. sigillatus* (a) and Expanded ^{13}C-NMR Spectra from 80 to 110 ppm (b)

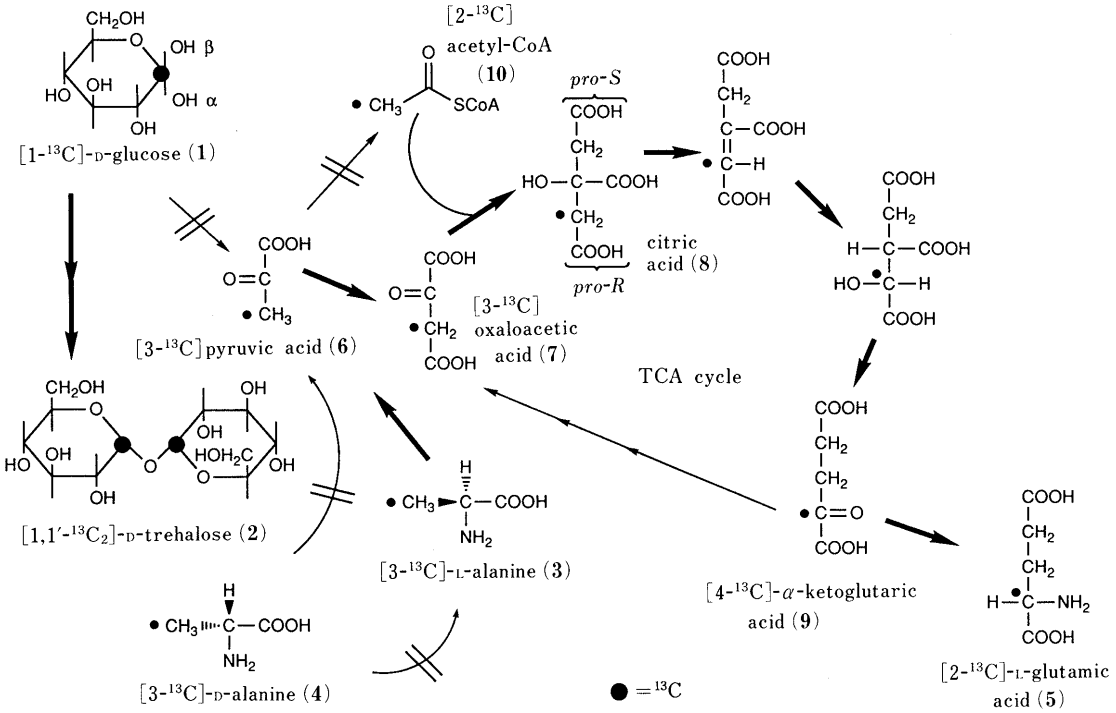

Fig. 2. The Metabolic Pathways of ^{13}C-Labeled Compounds in *G. sigillatus*

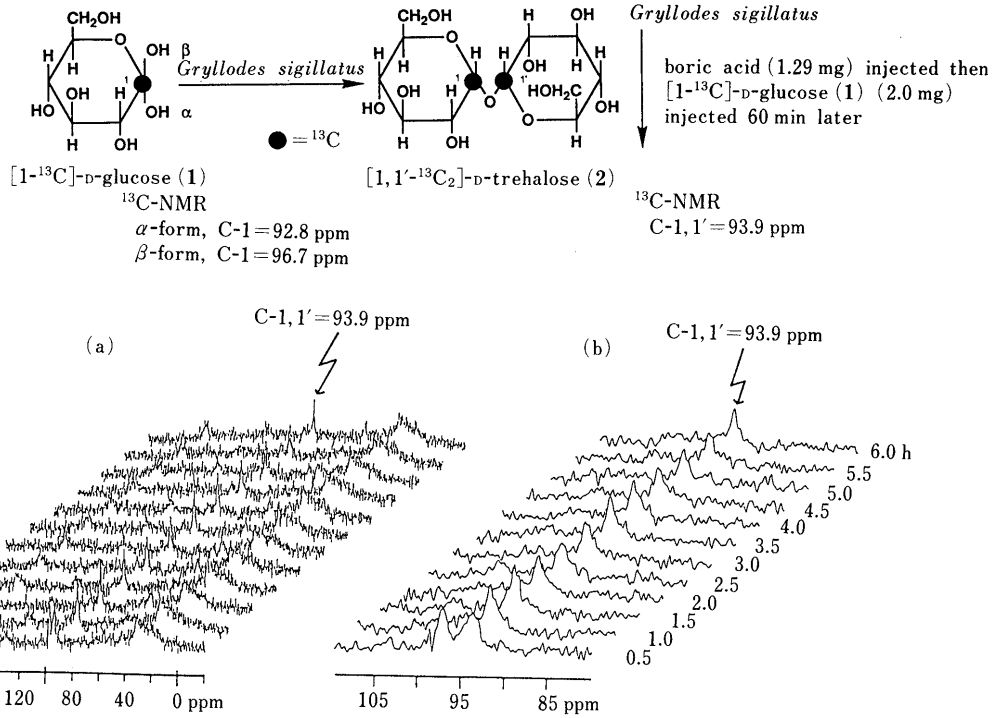

Fig. 3. ^{13}C-NMR Spectra Showing the *in Vivo* Transformation of [1-^{13}C]-D-Glucose (**1**) in the Presence of Boric Acid in *G. sigillatus* (a) and Expanded ^{13}C-NMR Spectra from 80 to 110 ppm (b)

processes and to decrease metabolism, usually is used as a antihelmintic for the cockroach. We investigated boric acid have effects upon the metabolism of D-glucose. When [1-^{13}C]-D-glucose (**1**) was intraabdominally injected into *G. sigillatus* 30 min after boric acid, the transformation of [1-^{13}C]-D-glucose (**1**) to [1,1'-$^{13}C_2$]-D-trehalose (**2**) was completed within 1 h (Fig. 3). Thus the metabolism of [1-^{13}C]-D-glucose (**1**) to [1,1'-$^{13}C_2$]-D-trehalose (**2**) unexpectedly was speeded up by boric acid.

Next, [3-^{13}C]-L-alanine (**3**) was intraabdominally injected into *G. sigillatus*. After 1 h, the C-3 signal of injected [3-^{13}C]-L-alanine (**3**) was observed at 17.9 ppm by ^{13}C-NMR *in vivo*.[3b-e,6] 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 from L-alanine *via* the Tricarboxylic acid (TCA) cycle (Fig. 4).[3b,c,6] The signal of [3-^{13}C]-L-alanine (**3**) finally disappeared. However, when [3-^{13}C]-D-alanine (**4**) was injected into *G. sigillatus*, it was not transformed

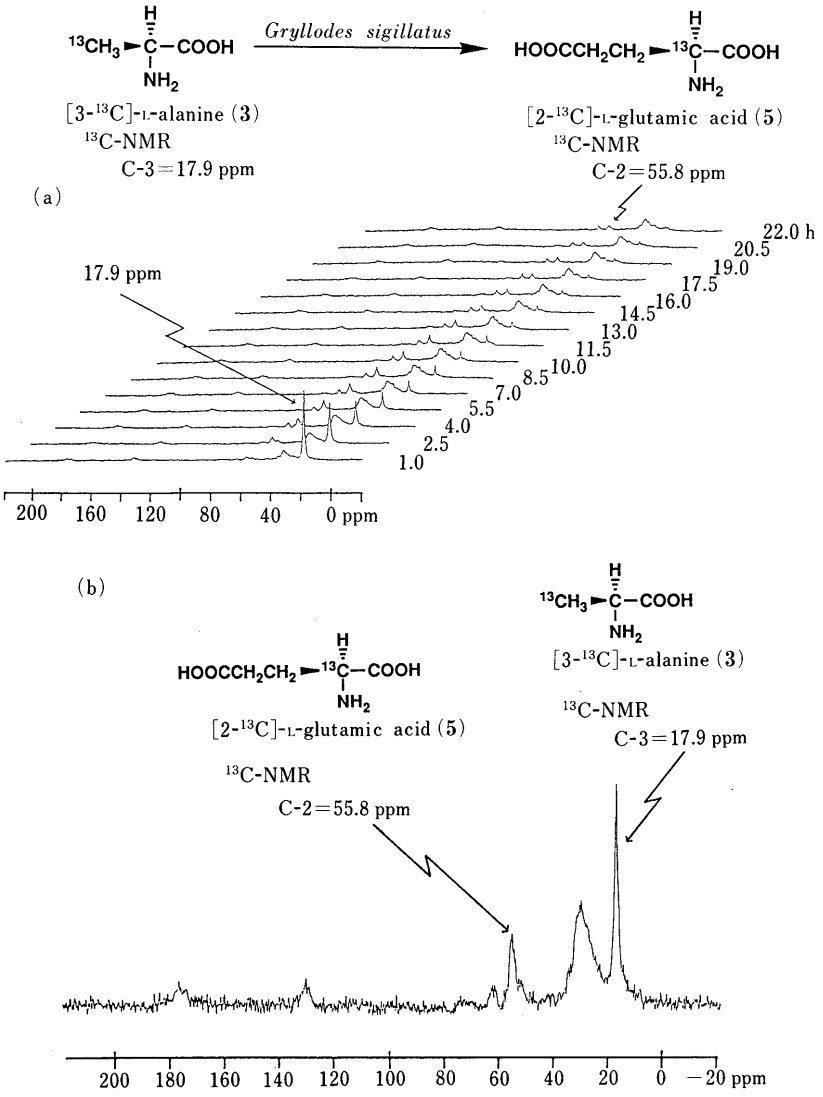

Fig. 4. ^{13}C-NMR Spectra Showing the *in Vivo* Transformation of [3-^{13}C]-L-Alanine (**3**) in *G. sigillatus* (a) and Expanded ^{13}C-NMR Spectrum at 4 h (b)

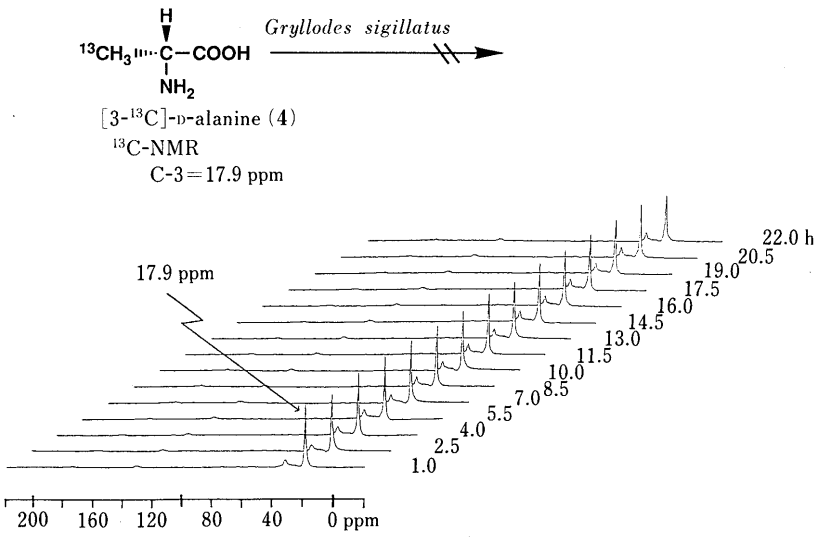

Fig. 5. ^{13}C-NMR Spectra Showing the Absence of *in Vivo* Transformation of [3-^{13}C]-D-Alanine (**4**) in *G. sigillatus*

(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-^{13}C]acetyl-Coenzyme A (**10**), which would lead successively to *pro-S* methylene ^{13}C-labeled citric acid, [2-^{13}C]-α-ketoglutaric acid, and finally [4-^{13}C]-L-glutamic acid (Fig. 2).

Experimental

Instruments ^{13}C-NMR spectra *in vivo* were recorded 100 MHz on a JEOL GSX-400 spectrometer with 10 mm multinuclear probes, referenced to $CDCl_3$ 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-^{13}C]-D-Glucose (99 atom% ^{13}C) was supplied by Cambridge Isotope Laboratories. [3-^{13}C]-D-Alanine[7] and [3-^{13}C]-L-alanine[7] (100% ee, 99 atom% ^{13}C) were prepared from ^{13}C-iodomethane (99 atom% ^{13}C), which was obtained from Cambridge Isotope Laboratories.

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

Measurement of Metabolism of [1-^{13}C]-D-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-^{13}C]-D-glucose (2.0 mg) in water (10 μl) was intraabdominally injected, and the insect was put into an NMR tube. The time course of signals was observed by ^{13}C-NMR as above.

Conclusion

The transformation of ^{13}C-labeled compounds was successfully monitored by *in vivo* ^{13}C-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 D-amino acid and L-amino acid was first observed, the metabolic pathways were confirmed for ^{13}C-labeled position of metabolite. This technique could be applicable to the studies of the metabolism of various drugs and agricultural chemicals.

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References and Notes

1) *a*) K. Kurumaya, T. Okazaki, N. Seido, Y. Akasaka, Y. Kawajiri, M. Kajiwara, and M. Kondo, *J. Labelled Comp. Radiopharm.*, **27**, 217 (1989); *b*) K. Kurumaya, T. Okazaki, and M. Kajiwara, *Chem. Pharm. Bull.*, **37**, 1151 (1989); *c*) K. Kurumaya, T. Okazaki, and M. Kajiwara, *Chem. Pharm. Bull.*, **38**, 1058 (1990).
2) *a*) G. R. Wyatt and G. F. Kalf, *J. Gen. Physiol.*, **40**, 833 (1957); *b*) D. J. Candy and B. A. Kilby, *Nature* (London), **183**, 1594 (1959); *c*) D. J. Candy and B. A. Kilby, *Biochem. J.*, **78**, 531 (1961).
3) *a*) E. Breitmaier and W. Voelter, "Carbon-13 NMR Spectroscopy," VCH Verlagsgesellschaft, Weinheim 1987, pp. 27, 380; *b*) K. Ugurbil, T. R. Brown, J. A. den Hollander, P. Glynn, and R. G. Shulman, *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 3742 (1978); *c*) K. L. Behar, O. A. C. Petroff, J. W. Prichard, J. R. Alger, and R. G. Shulman, *Magnetic Resonance in Medicine*, **3**, 911 (1986); *d*) B. J. Blackburn, H. M. Hutton, M. Novak, and W. S. Evans, *Exp. Parasitol.*, **62**, 381 (1986); *e*) C. A. Behm, C. Bryant, and A. J. Jones, *Int. J. Parasitol.*, **17**, 133 (1987).
4) T. Usui, N. Yamaoka, K. Matsuda, K. Tuzimura, H. Sugiyama, and S. Seto, *J. Chem. Soc., Perkin Trans. 1*, **1973**, 2425.
5) K. Iida and M. Kajiwara, *J. Chromatogr.*, **515**, 573 (1990). $^{12}C_{10}{}^{13}C_2H_{22}O_{11}$: molecular weight = 344.31. FAB-MS: $m/z = 343$ ($M^+ - 1$, 3%).
6) M. Barany, C. Arus, and Y-C. Chang, *Magnetic Resonance in Medicine*, **2**, 289 (1985).
7) K. Iida and M. Kajiwara, *J. Labelled Comp. Radiopharm.*, in press.