Communications to the Editor

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A ^{19}F NMR STUDY OF 2-DEOXY-2-FLUORO-D-GALACTOSE IN MICE

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The metabolic pathway of 2-deoxy-2-fluoro-D-galactose (FDGal) in mice was studied by ^{19}F NMR. Efficient accumulation of FDGal in liver was demonstrated by NMR, which is consistent with the results of Ishiwata et al. using radioactive ^{18}FDGal. The new discovery is that this fluorinated hexose was converted to 2-deoxy-2-fluoro-D-glucose (FDG) through UDP-FDGal and UDP-FDG apparently by the action of UDP-Gal epimerase.

KEYWORDS — F-19 NMR; mouse; liver; 2-deoxy-2-fluoro-D-galactose; 2-deoxy-2-fluoro-D-glucose; functional diagnosis

^{19}F NMR is a powerful tool for studying the metabolic pathway of 2-deoxy-2-fluoro-D-glucose (FDG) in mice.[1] It has long been explained that FDG is phosphorylated to FDG-6-phosphate (FDG-6-P) and the latter is subjected to metabolic trapping due to the presence of the F atom in place of the OH group at position 2.[2] However, our experiment has shown that FDG-6-P is further converted to FDM-6-P. This unexpected reaction is due to the action of glucose-6-P isomerase.[3] So it is of interest to find the metabolic pathway of FDGal. FDGal has been found to accumulate in the liver, mainly in the form of FDGal-1-P, changing gradually into UDP-FDGal, and is expected to be a potential reagent for diagnosis by Position Emission Tomography.[4] In this communication, we report the results of a ^{19}F NMR study of FDGal in mice. A wide variety of metabolites were found: FDG and FDG-1-P, FDG-6-P, and possibly UDP-FDG in addition to FDGal-1-P and UDP-FDGal.

FDGal was prepared by the method reported previously.[5] FDGal-1-P was prepared according to the methods of MacDonald,[6] and by the following method. FDGal (4 mmol/l) was incubated at 37 C for 2 h with 10.3 kU of galactokinase/l (Sigma G0130) in the presence of 3 mmol of magnesium acetate/l, 1 mmol of EDTA/l, 5 mmol of ATP-2Na/l, and 10 mmol of dithiothreitol/l in 150 mmol of triethanolamine-HCl/l buffer adjusted to pH 7.9 at 37 C for 2 h. UDP-FDGal was prepared by the method of Smith.[7]

Six-week-old male ddY mice (25 - 30g) were fasted for 16 h before FDGal injection. FDGal dissolved in isotonic saline was injected (0.15 g/kg) intravenously through the tail vein. The mice were sacrificed at 5 or 30 m and 3

or 24 h by cervical dislocation. The excised organs were heated at 100 C for 3 m in 10-mm od. NMR sample tubes to deactivate enzymes. The samples were kept frozen until NMR measurements.

^{19}F NMR spectra were taken on JEOL FX-100 and GSX-270 spectrometers at ambient temperature of 23 - 26 C. Hexafluorobenzene (0.25% in benzene[8]) was used as an external standard for chemical shift measurements. For the S/N improvement of dilute fluorine compounds in organs, the measurements were made under partial saturation conditions optimized for the typical T_1 of the metabolites. The concentration of fluorine compounds in tissue was obtained by the method of ref.1.

The proton-decoupled ^{19}F spectra of α-, and β-FDGal in aqueous solution gave signals at -44.5(α-FDGal), and -44.3 ppm (β-FDGal) from hexafluorobenzene. In both methods of FDGal-1-P synthesis, a strong signal of α-FDGal-1-P at -44.2 ppm and a weak one of -FDGal-1-P at -44.0 appeared with weak signals of reactants at -44.3 and -44.5 ppm. The reaction mixture of FDGal with galactokinase-galactose transferase[7] gave a strong signal at -44.2 ppm and a new peak at -45.1 ppm. The chemical shift and the coupling constants of the signal at -44.2 ppm indicate that this signal is due to FDGal-1-P. The signal at -45.1 ppm is assigned to UDP-FDGal.

The urine of FDGal-injected mice gave ^{19}F NMR signals with chemical shifts and coupling constants identical to those of FDGal and FDG. The formation of FDG in mice was confirmed. The ^{19}F NMR spectra of excised liver are shown in Fig. 1. Because of insufficient resolution, FDGal and FDGal-1-P are not resolved. New signals appeared at -45 and -37 ppm, increasing their intensities with time. Another signal appeared later at -36 ppm. The water extract of liver gave a high resolution spectra suitable for the identification of metabolites. (Fig. 1d).

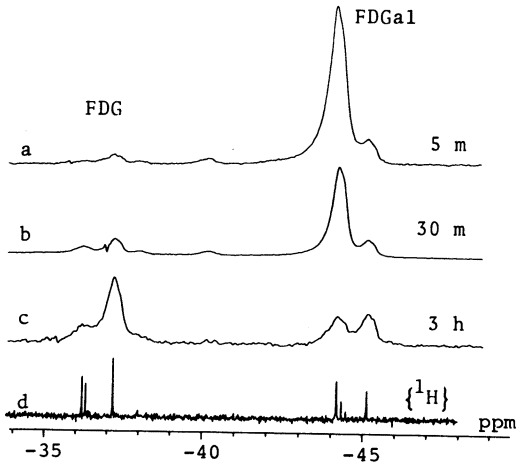
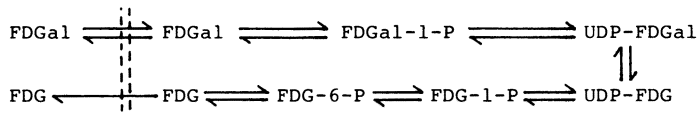


Fig. 1. ^{19}F NMR Spectra (254 MHz) of Excised Liver of FDGal-Injected Mouse
a - c, Excised liver; d, ^{1}H decoupled spectrum of liver extract.

Assignments can be made with reference to the spectral data of standard samples as follows. The signal at -36.2 ppm is β-FDG-6-P and/or β-FDG, the one at -36.3 ppm

is α-FDG and/or α-FDG-1-P, the one at -36.4 ppm is α-FDG-6-P.[1,3] The signal that appeared at -44.2 ppm is -FDGal-1-P, those of -44.3 and -44.4 ppm are α-, and β-FDGal, respectively, and the one at -45.1 ppm is UDP-FDGal. The strong signal at -37.2 ppm is tentatively assigned to UDP-FDG for the following reasons. First, UDP-FDGal appeared at a 1 ppm higher field than FDGal and FDGal-1-P. The one at -37.2 ppm is located at a field equally higher than FDG or FDG-1-P. Since the structural relations between FDGal and UDP-FDGal and between FDG and UDP-FDG are similar, it is reasonable to expect a similar chemical shift difference in these sets. Second, in the case of galactose, UDP-galactose is metabolized to UDP-glucose, glucose-1-P, then to glucose-6-P, and goes into glycolysis. The formation of FDG must be the result of such enzymatic action except that the presence of the F atom instead of the OH at position 2 keeps the reaction from proceeding beyond FDG-6-P.[1] Therefore, the formation of UDP-FDG is essential for the occurrence of FDG-1-P, FDG-6-P, or FDG in the system. The growth of the -37 ppm peak before the appearance of the -36 ppm signal shows that the former is the precursor of the latter. The third reason is that the signals at -45 and -37 ppm decreased their intensities while those at -44 and at -36 ppm increased during several hours after the excised raw liver was kept at ambient temperature. The total signal intensity of the FDGal group and that of the FDG group, respectively, remained unchanged throughout this observation. The observed spectral change must be due to the change of UDP-FDGal to FDGal-1-P caused by the action of transferase, and to the change of UDP-FDG to FDG-1-P caused by the action of pyrophospholyrase, under unaerobic conditions. Here, the assignment of UDP-FDG is also confirmed. Although we did not make a direct proof by the synthesis of UDP-FDG, the above evidence gives a strong basis for the assignment of the -37.2 ppm signal.

The mechanism of these metabolism is proposed as follows.



FDGal was trapped in the form of both the FDGal group (FDGal, FDGal-1-P, and UDP-FDGal) and the FDG group (FDDG, FDG-6-P, FDG-1-P, and UDP-FDG).

The amounts of fluorinated hexoses in some organs determined by the signal intensities are listed in Table I. As expected, the most efficient accumulation of

TABLE I. Uptake of FDGal in Mouse Organs

Time after injection/m	Uptake/dose %/g tissue		
	5	30	180
Liver	12 ± 1	17 ± 3	10 ± 1
Kidney	10 ± 1	9 ± 2	7 ± 2
Brain	1.5± 0.03	1.8± 0.7	2.2± 0.9
Heart	1.3± 0.3	1.3± 0.2	8.3± 0.2

(n = 2, 3)

fluorinated hexoses occurred in livers in which the activity of galactokinase is the highest. This is consistent with the results of ^{18}FDGal accumulation by Ishiwata et al.[4] In addition, the formation of FDG-related compounds is the most efficient in liver, which reflects the function of the organ in converting Gal to Glu. The efficiency of such a reaction may be used for the functional diagnosis of liver.

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8) 0.25% Hexafluorobenzene in benzene appears at a field 3.34 ppm lower than pure hexafluorobenzene. Chemical shift values in this report are shifted as much as those in references 1 and 3 where pure hexafluorobenzene is used as the shift standard.

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