Whole-body Distribution of ^{11}C -(4)-L-Aspartic Acid in RatsToshihiko NAKAMURA†, Masayoshi AKISADA
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Enzymatic synthesis of ^{11}C -(4)-L-aspartic acid was undertaken using commercially available wheat germ phosphoenolpyruvate carboxylase. Whole-body distribution of the radioactive compound in rats showed higher accumulation in the salivary gland, glandular stomach and the pancreas, as well as in the lungs. Within 60 minutes after intravenous injection of ^{11}C -(4)-L-aspartic acid, about 60% is removed as $^{11}\text{CO}_2$ by expiration, indicating that the carbon atom at the fourth position of the radioactive compound is easily subjected to decarboxylation.

Key Words: carbon-11-(4)-L aspartic acid, enzymatic synthesis,
whole-body distribution, autoradiogram, rat

1. Introduction

There are two methods for synthesis of radiopharmaceuticals, i.e. chemical synthesis and enzymatic synthesis. The advantages of the latter method to the former are as follows:

- ① The process is simpler and quicker.
- ② The latter has higher selectivity.
- ③ Since carrier-free agents can be used, very high specific radioactivity is usually obtained.

④ With chemical synthesis, a mixture of D and L form is produced, whereas, with enzymatic synthesis L form alone is produced. For the amino acids, such as glutamic acid^{1),2)}, glutamine^{1),2)} and asparagine³⁾, ^{15}N -labeled compounds are made by enzymatic synthesis, while the ^{11}C -labeled-aspartic acid are synthesized using phosphopyruvate carboxylase obtained from chicken liver⁴⁾. It has been reported that ^{14}C -(U)-aspartic acid (Asp) specifically accumulated in the lung⁵⁾.

In the present study, enzymatic synthesis of ^{11}C -(4)-L-aspartic acid (^{11}C -Asp) was undertaken using commercially available phospho-

enolpyruvate carboxylase obtained from wheat germ. Using the obtained compound, its organ distribution and the excretion of $^{11}\text{CO}_2$ by expiration were examined in order to determine the feasibility as a scanning agent.

2. Experimental Methods

2.1 Radioactive compounds

Biosynthesis of ^{11}C -Asp was undertaken with two kinds of enzymes, i.e. phosphoenolpyruvate carboxylase (PEP carboxylase) and glutamic oxaloacetic transaminase (GOT). ^{11}C was labeled at the fourth position of Asp (Fig. 1).

$^{11}\text{CO}_2$ was produced by $^{14}\text{N}(p, \alpha)^{11}\text{C}$ reaction

Fig. 1 (A) Enzymatic synthesis of ^{11}C -Asp.(B) Chemical structure of ^{11}C -Asp.

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with N_2 gas as the target using the Baby Cyclotron at the National Sanatorium Nakano Hospital. An aqueous NaOH solution in which $^{14}CO_2$ was trapped was added to 2 ml of a buffered-enzyme solution (pH 8.0). The contents of the buffered solution were as follows: 10 μ moles phosphoenolpyruvate (PEP), 30 μ moles $MgCl_2$, 30 μ moles glutathione (GSH), 30 μ moles glutamate (Glu), 2.5 U of PEP carboxylase (Boehringer, Mannheim), and 20 U of GOT (Boehringer, Mannheim). The mixture was incubated for 20 minutes at 30°C. The resulting products were purified by absorption to IR-120 ion-exchanging column, and elution with 2 N ammonium hydroxide.

Thin-layer chromatography was used for examining radiochemical purity of the ^{14}C -Asp. The developing solvent I and II was ethanol : water (4 : 1) and butanol : acetic acid : water (3 : 1 : 1) respectively.

2.2 Animal experiments with ^{14}C -Asp and ^{14}C -Asp

2.2.1 Blood clearance of ^{14}C -Asp

Wister rats (weighing 140~200 g) were received a 2.77~20.7 MBq (75~560 μ Ci) of ^{14}C -Asp by injection into the tail vein. Whole blood was taken 1, 2, 3, 4, 5, 7, 10, 15, 20, 30, 45, 60, 90 and 120 minutes after the injection and radioactivity was measured using a well-type gamma-counter. The mean value of four rats was obtained.

2.2.2 Whole-body distribution of ^{14}C -Asp

Using Wister rats (weighing 95~255 g), a 1.85~22.2 MBq (50~600 μ Ci) of ^{14}C -Asp was injected into the tail vein. The animals were decapitated after 5, 10, 20 or 30 minutes and the weight of their organs, such as the lung, liver, kidney, spleen and pancreas was measured respectively. Radioactivity of these organs was measured using a well-type gamma-counter. Mean value of five animals in a group was obtained.

2.2.3 Excretion of ^{14}C -CO₂ by expiration

To Wister rats (weighing 90~105 g), a 10.1~25.9 MBq (300~700 μ Ci) of ^{14}C -Asp was injected into the tail vein. Expired ^{14}C -CO₂ was trapped as calcium carbonate at an interval of five minutes. Radioactivity was determined by a well-type gamma-counter until 40 minutes

after injection.

2.2.4 ^{14}C -Asp scintigraphy

188.7 MBq (5.1 mCi) of ^{14}C -Asp was infused into the rabbit ear vein, and the scintigrams were obtained at five minutes and 30 minutes after injection by an Anger camera attached with a specially manufactured Beattie type collimator.

2.2.5 Autoradiogram of rat

Wistar rats were injected with 27.0 kBq (0.73 μ Ci) of ^{14}C -Asp (by RCC Amersham), into the tail vein. After five and 60 minutes, the animals were sacrificed by ether anesthesia to prepare autoradiograms.

2.2.6 Excretion of ^{14}C -Asp by expiration

^{14}C -(4)-Asp or ^{14}C -(U)-Asp (RCC Amersham), of 27.0 kBq (0.73 μ Ci) or 33.3 kBq (0.90 μ Ci), respectively, was injected into the rat tail vein and $^{14}CO_2$ expired was measured continuously for over one hour with a liquid-scintillation radiorespirometer⁹.

3. Results

3.1 Enzymatic synthesis of ^{14}C -Asp

Using 2.17 GBq (58.6 mCi) of $^{14}CO_2$, maximal 188 MBq (5.1 mCi) of ^{14}C -Asp could be synthesized. The time required for the preparation was 50~70 minutes.

No radioactivity was detected in any fractions other than ^{14}C -Asp by thin-layer chromatography using solvent I and II.

3.2 Blood clearance of ^{14}C -Asp

Figure 2 shows the blood clearance curve of ^{14}C -Asp. The disappearance curve was biphasic, the first phase being rather rapid with a half-life of 80 seconds, while the half-life for the second phase was 50 minutes. After 10 minutes, almost 90% of ^{14}C -Asp disappeared from the blood.

3.3 Whole-body distribution of ^{14}C -Asp

Figure 3 shows the whole-body distribution of radioactivity in each organ at 5, 10, 20 and 30 minutes. From this figure, the accumulation in the spleen was about 3 times that in the blood five minutes after the injection, but it disappeared rapidly thereafter. The activity in the lung became maximum at about 10 minutes after the injection, whereas in the kidney and liver, the initial activity was high, with a

Fig. 2 Blood clearance curve of ^{14}C -Asp. Relative blood activity means the blood count ratio to one minute blood count after i.v. injection.

Fig. 3 Organ distribution of ^{14}C in rats after i.v. injection of ^{14}C -Asp. Relative organ activity means the organ count ratio to 5 minutes blood count after i.v. injection.

tendency to decrease gradually. The activity in the pancreas showed an U-shape curve and remained relatively high throughout compared with the other organs after 30 minutes.

Fig. 4 Removal of ^{14}C by expiration from rats after i.v. injection of ^{14}C -Asp.

3.4 Excretion of ^{14}C - CO_2 by expiration

Figure 4 showed the amount of expired $^{14}\text{CO}_2$ every 5 minutes which was expressed as a percentage of the intravenously injected dose. Activity of expired $^{14}\text{CO}_2$ reached a peak at 10~15 minutes after the injection of ^{14}C -Asp. This agreed well with the body distribution in the lung, where radioactivity reached a peak at 10 minutes after injection of ^{14}C -Asp, indicating that the radioactivity in the lung was mainly reflected by expired $^{14}\text{CO}_2$. About 39% of the initial dose was expired within 15 minutes and it reached to 68% within 40 minutes from the beginning.

3.5 Scintigram of ^{14}C -Asp

Figure 5 showed the frontal view of the scintigram of a rat 5 and 30 minutes after injection of ^{14}C -Asp. High activity was noticed in the head and neck, lung, liver, kidney and extremities. Little difference was seen between the 5 and 30 minutes. No significant activity was found in the pancreas.

3.6 Autoradiogram of ^{14}C -(4)-Asp

Figure 6 showed autoradiograms made at 5 and 60 minutes after injection. Table 1 showed the relative ^{14}C -(4)-Asp density of each organ to liver determined by microdensitometer (SMS 2405 B type) at 5, 10, 30 and 60 minutes after injection. For comparison, organ to liver

Fig. 5 Whole-body imaging of ^{11}C -Asp in rabbits after i.v. injection of ^{11}C -Asp.

Fig. 6 Whole-body autoradiograms of rats after i.v. injection of ^{14}C -Asp.

ratio of radioactivity at 30 minutes after injection of ^{11}C -Asp were also shown.

Intense radioactivity of ^{14}C -Asp was observed in the salivary gland and glandular stomach. High values were also noted in the lung, kidney and pancreas after five minutes. Relative density of pancreas to liver was increased with time.

Relative activity of each organ to liver of ^{11}C -Asp agreed well with that of autoradiogram

Table 1 Comparison of organ to liver ratio of the radioactivity in various organs after intravenous injection of ^{14}C -Asp or ^{11}C -Asp. ^{14}C -Asp ratio and ^{11}C -Asp ratio show the autoradiographic density ratio and counting ratio respectively.

Organ (min)	Organ to liver ratio				
	^{14}C -Asp			^{11}C -Asp	
	5	10	30	60	30
Lung	1.4	2.3	1.3	0.8	1.1
Spleen	1.0		0.6	0.8	0.6
Adrenal	0.9		0.6	1.5	0.6
Kidney	1.8		0.9	1.6	1.0
Pancreas	1.0		1.4	2.0	1.3
Glandular stomach	3.0		3.1	2.7	
Salivary gland	3.5		4.4	2.8	

of ^{14}C -(4)-Asp both obtained after 30 minutes injection.

3.7 Excretion of ^{14}C - CO_2 by expiration

Figure 7 showed an example of expired curves of $^{14}\text{CO}_2$ from rats given ^{14}C -(4)-Asp or ^{14}C -(U)-Asp expectively. Decarboxylation started immediately after injection and reached each peak within 10 minutes. It was noted that net counts of ^{14}C -(4)-Asp of the expiration peaks was higher than that of ^{14}C -(U)-Asp, although the former injected activity was less.

The relative expired dose of $^{14}\text{CO}_2$ within 60 minutes after injection, was $57.7 \pm 4.8\%$ for ^{14}C -(4)-Asp and $39.4 \pm 2.6\%$ for ^{14}C -(U)-Asp ($p < 0.05$). As the relative amount of $^{14}\text{CO}_2$

Fig. 7 Expiratory $^{14}\text{CO}_2$ curves of rats, after i.v. injection of ^{14}C -(4)-Asp and ^{14}C -(U)-Asp respectively.

Table 2 Comparison of the synthetic methods of ^{11}C -labeled amino acids

Synthetic method	Product	Precursor	Synthetic time (min)	Radiochemical yield (%)	
Chemical synthesis	Tryptophan ⁷⁾	H^{11}CN	40	30~40	DL form
	Valine ⁸⁾	H^{11}CN	45	70	"
	[3- ^{11}C]-Alanine ¹⁰⁾	$^{11}\text{CH}_3\text{I}$	50~60	50	"
	Methionine ¹¹⁾	$^{11}\text{CH}_3\text{I}$	80~100	90~98	"
	[1- ^{11}C]-Alanine ¹⁰⁾	$^{11}\text{CO}_2$	20	11	"
	Phenylalanine ¹³⁾	$^{11}\text{CO}_2$	40	24	"
	Phenylglycine ¹³⁾	$^{11}\text{CO}_2$	60	3	"
Enzymatic synthesis	^{11}C -(4)-Asp (our study)	$^{11}\text{CO}_2$	50~70	44~83	L-form

from ^{11}C -(4)-Asp was significantly higher than that of ^{11}C -(U)-Asp, the carbon in the fourth position of Asp seems to be easily decarboxylated. The amount of decarboxylated ^{11}C -Asp was almost the same to that found for ^{11}C -Asp.

4. Discussion

4.1 Enzymatic synthesis of ^{11}C -Asp

For the preparation of ^{11}C -labeled amino acids, a synthetic method using $^{11}\text{CO}_2$, $^{11}\text{CH}_3\text{I}$, and H^{11}CN as precursors has been adopted. In the case of tryptophan⁷⁾ and valine^{8),9)}, $^{11}\text{CO}_2$ is first made into H^{11}CN , and then DL- ^{11}C -amino acid by labeling the carboxylic acid in the first position by the Bücher-Strecker's reaction. $^{11}\text{CH}_3\text{I}$, made from $^{11}\text{CO}_2$, was incorporated to [3- ^{11}C]-alanine¹⁰⁾, and [S- $^{11}\text{CH}_3$]-methionine^{11),12)}. Further, with regard to [1- ^{11}C]-alanine¹⁰⁾, -phenylalanine^{13),14)} and -phenylglycine^{13),14)}, carboxylic acid in the first position was labeled directly with $^{11}\text{CO}_2$.

Enzymatically synthesized ^{11}C -Asp was compared with other amino acids regarding their precursor, synthetic time and radiochemical yield (Table 2).

(1) Radiochemical yield of ^{11}C -Asp by the enzymatic synthesis was believed to be higher than the yields of other amino acids by the chemical syntheses. Precursor H^{11}CN and $^{11}\text{CH}_3\text{I}$ was prepared from $^{11}\text{CO}_2$ respectively. Radiochemical yield of ^{11}C -(4)-Asp by enzymatic synthesis was higher than those of alanine, phenylalanine and phenylglycine by chemical synthesis, although all of the above radiopharmaceuticals were synthesized by the same $^{11}\text{CO}_2$ precursor.

(2) Enzymatic synthesis is an easy process

made of just mixing raw materials and incubating them.

(3) ^{11}C -Asp is obtained as a carrier-free product.

(4) By enzymatic synthesis, only L-form Asp was obtained. D-form degrades the image quality as it increases the background.

From the forgoing results, enzymatic synthesis is considered to be superior to the conventional chemical synthesis.

4.2 Whole-body distribution

Masuoka, et al.⁵⁾ showed a marked accumulation of ^{11}C -(U)-Asp in the lung, salivary gland, pancreas, liver and kidney of the mouse with the autoradiograms 5 minutes after injection.

In the present study, similar results were obtained with the autoradiogram 5 minutes after intravenous injection of ^{11}C -(4)-Asp and these high accumulation persisted for one hour after the injection. According to Busch, et al.¹⁵⁾, however, the accumulation of Asp in the pancreas was less than that of other amino acids. Indeed, the comparison of ^{11}C -Asp with other ^{11}C -labeled amino acids revealed the results indicating little accumulation of ^{11}C -Asp in the pancreas.

When ^{11}C -Asp was given to rabbits, the Anger scintillation-camera failed to obtain the pancreatic imaging, although positron image could not be obtained in this experiment.

4.3 Excretion by expiration

The carbon atoms at the first and fourth position of Asp is easily subjected to decarboxylation compared to the carbon atoms in other positions. This fact can be explained on the

basis of the trichloroacetic acid (TCA) cycle. That is, Asp is subjected to deamination by GOT, becoming oxaloacetic acid, and enters the TCA cycle. In the cycle, carbon atoms at the first and fourth position of Asp are decarboxylated. Accordingly, the Asp labeled at the fourth carbon is more easily decarboxylated than the Asp universally labeled. In the present study, $^{14}\text{CO}_2$ from ^{14}C -(4)-Asp revealed a significant higher volume when compared with that from the ^{14}C -(U)-Asp. It was further detected that about 60% of the radioactivity injected in the form of ^{14}C -(4)-Asp was expired as $^{14}\text{CO}_2$ within one hour. In considering the fact that the accumulation of ^{14}C -(4)-Asp in the lung was maximal after 10 minutes, as shown also by the autoradiograms, it can be understood that the radioactivity in the lung is that of $^{14}\text{CO}_2$. The same condition can be applied to the experiment using ^{14}C -Asp. With ^{14}C -Asp, the accumulation in the lung reached a peak within 10 minutes and the expired $^{14}\text{CO}_2$ reached a peak 10~15 minutes after injection. In other word, ^{14}C activity existed in the lung was attributed to $^{14}\text{CO}_2$.

From these findings it is established that the labeled carbon at the fourth position of ^{14}C -Asp is extremely easily subjected to metabolism, and that the rate of metabolism can be observed by $^{14}\text{CO}_2$ expiration. Consequently, this phenomenon may be possible to apply to the liver function test.

Glaubitt and Hampel¹⁶⁾ reported that the volume of $^{14}\text{CO}_2$ expired after injection of ^{14}C -labeled valine and histidine to patients with hepatic cirrhosis was less than expired $^{14}\text{CO}_2$ from normal subjects. Regarding ^{14}C -Asp, it may be also possible to determine amino acid metabolism indirectly in patients with liver disorders by measuring the amount of $^{14}\text{CO}_2$ expired after injection of ^{14}C -Asp. Although ^{14}C is one of the ultra-short half-life positron emitters, the scintigraphy of the lung could be obtained.

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要 旨

酵素合成法により調製した ^{11}C -L-アスパラギン酸の体内分布中村俊彦[†], 秋貞雅祥, 重松昭世*

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新しいアミノ酸代謝診断薬として ^{11}C -(4)-L-アスパラギン酸の酵素合成を行った。ラットにおける体内分布では、唾液腺、腺胃、脾臓ならびに肺に集積が多かった。一方、静注60分で約60%が呼気中に CO_2 ガスとして排泄され、 ^{11}C -(4)-L-アスパラギン酸の4位の炭素原子は脱炭酸を受けやすいことが分かった。

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