Increased Amounts of D-Enantiomer Dependent on Alkaline Concentration in the Synthesis of L-[methyl-11C] Methionine

III. 8 Increased Amounts of D-Enantiomer Dependent on Alkaline Concentration in the Synthesis of L-[methyl-^{11}C] Methionine

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Positron emission tomography (PET) using positron-emitting radiopharmaceuticals has potential for measuring quantitatively amino acid utilization or protein synthesis rates in vivo. Since Comar and co-workers synthesized L-[methyl-^{11}C]methionine by reaction of L-homocysteine thiolactone and [^{11}C]CH_3I,[1] several other groups employed this reaction.[2-4] The method is assumed to give pure L-enantiomer. In this communication we report on the percentage of D-enantiomer contaminant in L-[methyl-^{11}C]methionine prepared from L-homocysteine thiolactone and [^{11}C]CH_3I.

Materials and Methods

To prepare L-[methyl-^{11}C]methionine, after trapping of [^{11}C]CH_3I in 1 mL of acetone, 0.5 mL of water containing 3.0 mg of L-homocysteine thiolactone (Sigma Chemicals) and 0.5 mL of 0.1 M NaOH were added to the acetone solution. For the preparation of D,L-[methyl-^{11}C]-methionine D,L-homocysteine thiolactone was used as precursor. The reaction mixture was heated at 60°C for 10 min. After removal of unreacted [^{11}C]CH_3I with a flow of helium, the reaction mixture was evaporated to dryness. The residue was dissolved in saline, and the solution was neutralized with 0.1 M HCl. For studying the effect of the reaction conditions on the proportion of D-enantiomer in L-methionine preparations, the [^{11}C]CH_3I in acetone was divided into three equal parts, and the reaction was carried out at one third of the scale used in the above method: varying concentrations of 0.025 M, 0.25 M and 1.0 M NaOH in 50% aqueous acetone were used. Several different reaction temperatures, 40°C, 60°C and 80°C, and different reaction times, 2, 5 and 10 min, were also compared. After removal by a flow of helium of unreacted [^{11}C]CH_3I, a small amount (up to 10 μL) of the reaction mixture was applied directry, without removal of acetone and neutralization, on the HPLC column for enantiomeric separation of methionine.

Enantiomeric analysis was carried out by HPLC.[5] A reverse-phase column, NVC18, was used. The column was eluted with 0.03 M aqueous sodium acetate containing 0.017 M L-proline and 0.008 M cupric acetate at a flow rate of 2 mL/min.

The radiochemical purity of ^{11}C-labeled methionine was also analyzed by HPLC. A Partisil-10-SCX column (4.6 mm i.d. × 25 cm, Whatman) was used with 0.1 M potassium phosphate, pH 2.6, as eluent at a flow rate of 2.0 mL/min. The retention time of methionine was 2.9 min.

Results and Discussion

In the analysis of L- and D,L-[methyl-^{11}C]methionine preparations on the SCX column, more than 99% of radioactivity was detected in a peak corresponding to methionine. Fig. 1 represents the HPLC-elution patterns of L- and D,L-[methyl-^{11}C]methionine preparations using the reverse-phase column and the chiral mobile phase. The radioactive peak with a retention time of 5 min was identified to be D-enantiomer. The L-form had a retention time of 12 min.

The ratio of D-enantiomer in L-[methyl-^{11}C]methionine preparations was measured in relation to different reaction conditions: concentration of NaOH, reaction temperature and different batches of L-homocysteine thiolactone (Table 1). The radiochemical yield was better than 70% under the various reaction conditions investigated. The D/L ratio increased with increasing concentrations of NaOH. No substantial effect on the ratio was observed for different reaction temperatures between 40°C and 80°C, nor when different lots of L-homocysteine thiolactone were used. From Table 2 it can be concluded that the ratio of D-enantiomer did not vary with reaction time, and that L-[methyl-^{11}C]methionine once prepared is not converted to D-form using 1.0 M aqueous NaOH for 30 min. Under these reaction conditions a few percent of ^{11}C with a retention time of 2 min on the reverse-phase column was observed because of decomposition of methionine.

Our results demonstrate that L-enantiomer is the main product from the reaction of L-homocysteine thiolactone and [^{11}C]CH_3I, and that racemization of L-[methyl-^{11}C]methionine under the reaction conditions used is negligible. Since at the lowest concentration of NaOH used the percentage of measured D-enantiomer is still 2.1% (average of 9 runs), it is possible that a small amount of D-homocysteine thiolactone is present as an impurity in commercially purchased L-enantimor. Differences in reactivity between D- and L-enantiomers are not likely. The increased amounts of D-enantiomer dependent on NaOH concentration possibly derive from the rearrangement of a chiral center during the reaction. The used NaOH concentrations reported in the literature are 0.15 M[1] and 0.2 M.[2,3] Under these reaction conditions only 2% to 4% of D-enantiomer is supposed to be present as contaminant in the L-[methyl-^{11}C]methionine preparation.

It is not expected that a small percentage of enantiomeric impurity in L-[methyl-^{11}C]methionine preparations will reduce the significance of L-[methyl-^{11}C]methionine for the clinical evaluation of patients with tumors or with brain disorders. The contamination of D-[methyl-^{11}C]methionine does not reduce tumor or brain uptake.[6] Notwithstanding the clinical usefulness of L-[methyl-^{11}C]-methionine[7], this labeled amino acid poses, because of a

complicated metabolic fate[8,9], an inherent problem for kinetic modeling to measure quantitatively protein synthesis rate in vivo. The significance of L- methyl-^{11}C methionine should be its applications in the methionine utilization or methionine extraction.

References

1) Comar D., Cartron J. -C., Maziere M. and Marazano C., Eur. J. Nucl. Med. 1 (1976) 11.
2) Berger G., Maziere M., Knipper R. et al., Int. J. Appl. Radiat. Isot. 30 (1979) 393.
3) Meyer G.-J., Osterholz A. and Hundeshagen., J. Label. Compd. Radiopharm. 19 (1982) 1286.
4) Kubota K., Yamada K., Fukuda H. et al., Eur. J. Nucl. Med. 9 (1984) 136.
5) Gil-Av E., Tishbee A. and Hare P. E., J. Am. Chem. Soc. 102 (1980) 5115.
6) Schober O., Duden C., Meyer G.-J. et al., Eur. J. Nucl. Med. 13 (1987) 103.
7) Kubota K., Matsuzawa T., Ito M. et al., J. Nucl. Med. 26 (1985) 37
8) Ishiwata K., Ido T., Abe Y. et al., Nucl. Med. Biol. 15 (1988) 123.
9) Ishiwata K., Vaalburg W., Elsinga P. H. et al., J. Nucl. Med. (in press).

Table 1. Effect of NaOH concentration, reaction temperature and different lots of L-homocysteine thiolactone on the percentage of D-enantiomer in L-[methyl-^{11}C]methionine preparation.

NaOH (M)	L-Homocysteine thiolactone lot No	Reaction temperature 40°C	60°C	80°C
0.025	1		1.9%	
	2	1.6%	2.1%, 2.2%	2.7%
	3		2.2%	
			(1.8%-2.8%)**	
0.25	1	4.3%	3.0%, 4.1%	3.6%
	2		3.6%	
	3		2.6%	
1.0	1	9.2%	7.1%, 8.3%	8.4%
	2		6.3%	
	3		6.9%	
0.25	D,L-HCTL*		50.4%	
			(49.1%-52.7%)***	

Reaction time: 10 min

* D,L-[methyl-^{11}C]methyonine was synthesized using D,L-homocysteine thiolactone.

Data represent the results of each run, with averages of four runs** and three runs*** with range in parentheses.

Table 2. Effect of reaction time and NaOH concentration on the percentage of D-enantiomer in L-[methyl-^{11}C]methionine.

NaOH (M)	L-Homocoysteine Thiolactone lot No	Reaction time 0 min	2 min	5 min	10 min	30 min
0.25*	2		3.8%	3.8%	3.6%	
1.0*	3		6.3%	6.1%	6.9%	
1.0	^{11}C-Met**	2.1%			2.3%	1.9%

* The reaction was carried out in 50% aqueous acetone at 60°C.

**L-[methyl-^{11}C]methionine, synthesized by reaction of L-homocysteine thiolactone (lot No 2) in 0.025 M NaOH for 10 min at 60°C. The amino acid prepared was heated in 1.0 M aqueous NaOH at 60°C for 10 min or 30 min.

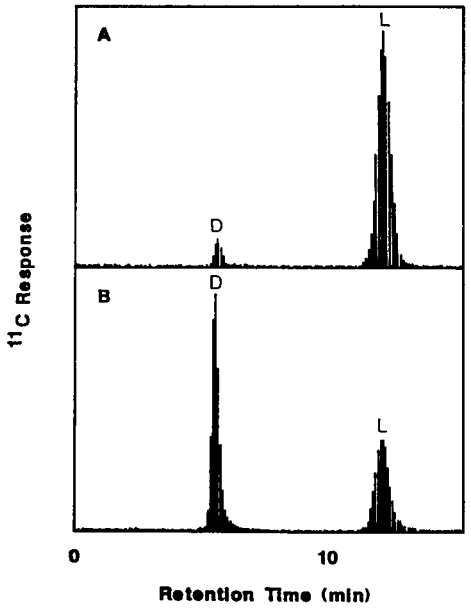

Fig. 1. Radio-high performance liquid chromatographic analysis of ^{11}C-labeled methionines prepared from L-homocysteine thiolactone (A) and from D,L-homocysteine thiolactone (B).