RESOLUTION OF [^{11}C]DL-LEUCINE AND [^{11}C]DL-TRYPTOPHAN BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Lee C. Washburn*, Tan Tan Sun*, Billy L. Byrd*, and Alvin P. Callahan†
*Medical and Health Sciences Division, Oak Ridge Associated Universities, and †Nuclear Medicine Group, Oak Ridge National Laboratory, Oak Ridge, TN 37831 U.S.A.

SUMMARY

High-performance liquid chromatographic (HPLC) resolution of [^{11}C]DL-leucine and [^{11}C]DL-tryptophan, using modifications of a previously developed technique for resolution of [^{11}C]DL-valine, was used to produce [^{11}C]L-leucine and [^{11}C]L-tryptophan. The technique employs commercially available reverse phase HPLC columns and chiral mobile phases containing cupric acetate and L-proline.

Key Words: [^{11}C]DL-Leucine, [^{11}C]DL-Tryptophan, High-performance liquid chromatography, Optical resolution, [^{11}C]L-Leucine, [^{11}C]L-Tryptophan

INTRODUCTION

Most methods for the synthesis of ^{11}C-labelled amino acids, including our modified Bücherer-Strecker technique (1,2), produce racemic mixtures. However, physiological studies, such as positron tomographic measurement of regional cerebral protein synthesis (3) and evaluation of organ function (4), require natural L-enantiomers. Therefore, development of methods for the separation of the L-enantiomers from ^{11}C-labelled amino acid racemates that are compatible with the short $T_{1/2}$ of carbon-11 (20.4 min) is necessary. Three methods have been reported: 1. Immobilized amino acid oxidases which utilize the undesired enantiomer as a substrate (5, 6); 2. Affinity chromatography using human serum albumin coupled to a sepharose resin (specific for [^{11}C]DL-tryptophan) (7); and 3. High-performance liquid chromatography (HPLC) using a chiral mobile phase (8). We have previously reported the HPLC technique for resolution of [^{11}C]DL-valine (8). This report describes the modification of the method to make it applicable to the resolution of [^{11}C]DL-leucine and [^{11}C]DL-tryptophan. [^{11}C]L-Leucine and [^{11}C]L-tryptophan are potential radiopharmaceutical agents

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for positron tomographic measurement of regional cerebral protein synthesis and differential diagnosis of pancreatic disease, respectively.

MATERIALS AND METHODS

Our HPLC system (LC/System Support Unit I, including Model 2396-89 Milton Roy Instrument duplex miniPump and Rheodyne Model 7120 syringe-loaded sample injection valve, Laboratory Data Control, Riviera Beach, FL) was fitted with a 2.0 mL sample loop. For resolution of [^{11}C]DL-leucine, we used a preparative (10 mm i.d. X 25 cm length) Ultrasphere ODS, 5 μm column (Altex Scientific, Inc., Berkeley, CA). However, for [^{11}C]DL-tryptophan, the copper-amino acid complexes would not elute from the Ultrasphere ODS column; therefore, a Spherisorb ODS, 5 μm column (Applied Science Division, Milton Roy Company Laboratory Group, State College, PA) of the same dimensions was used. The columns were equilibrated with the appropriate mobile phase, which was degassed under vacuum before use. For [^{11}C]DL-leucine, the mobile phase consisted of 0.017 M L-proline and 0.008 M cupric acetate in 0.03 M aqueous sodium acetate; for [^{11}C]DL-tryptophan, the mobile phase was produced by adding ethanol to this same solution to a concentration of 15% by volume, which increases the solubility of the copper-amino acid complexes and consequently increases their elution velocities. The flow rate was adjusted to 7.2 mL/min and 5.0 mL/min for resolution of [^{11}C]DL-leucine and [^{11}C]DL-tryptophan, respectively.

Crude ^{11}C-carboxyl-labelled DL-leucine and DL-tryptophan were synthesized at the Oak Ridge National Laboratory's 86-inch cyclotron complex as previously described (2), with the following exceptions: (1) The gas absorption column was not rinsed with water; (2) the amount of NaOH used for hydrolysis was reduced from 1.0 mL to 0.7 mL of 6.25 N NaOH; and (3) the carrier potassium cyanide was reduced to 1 mg for [^{11}C]DL-leucine and to 0.01 mg for [^{11}C]DL-tryptophan. The crude reaction mixture was combined with 9.6 mg of cupric acetate and 11.7 mg of L-proline in 0.1 mL of water, and the pH of the solution was adjusted to 5-6 using concentrated HCl. The resulting solution was passed through a 0.22 μm filter and injected onto the HPLC column.

Elution of the ^{11}C-labelled mixture was monitored using a shielded gamma probe. The fraction containing the ^{11}C-labelled L-enantiomer was acidified by adding 2 mL of 6 N HCl. In the case of tryptophan, the acidified solution was filtered and the filtrate was loaded directly onto a 1.0 x 15 cm, AG 50W-X2, 50-100 mesh cation-exchange bed in the hydrogen form (Bio-Rad Laboratories, Richmond, CA), which had been previously washed with water and then 1 N HCl. For leucine the acidified L-fraction was combined with 3 mL of a saturated aqueous solution of hydrogen sulfide (made fresh that day) to precipitate the copper as cupric sulfide. The mixture was filtered and the filtrate was loaded onto the cation-exchange column as above. After loading, the column was washed once with 100 mL of 1 N HCl and twice with 50 mL of water. The ^{11}C-labelled L-amino acid was then eluted with 0.2 N NaOH. Final radiopharmaceutical processing (neutralization, microfiltration, and pyrogen testing) was carried out as described previously (2).

In the development of the resolution method, experiments were carried out using tracer amounts of carbon-11 and carbon-14 activity under conditions that would simulate the resolution of a full batch of crude ^{11}C-labelled racemic amino acid, as described previously for resolution of [^{11}C]DL-valine (8).

The level of L-proline in the purified [^{11}C]L-tryptophan solution was determined by spiking the ^{11}C-labelled L-amino acid fraction from the HPLC with 5 μCi (0.02 μmole) of ^{14}C-labelled L-proline (ICN Chemical and Radioisotope Division) prior to final cation-exchange purification. Carbon-14 assays of the wash solutions and the ^{11}C-labelled L-amino acid fraction were carried out, after decay of the carbon-11, by standard liquid scintillation counting techniques. Copper levels in the purified L-amino acid solutions were determined by use of tracer levels of copper-64 (Oak Ridge National Laboratory) or analysis by an independent commercial laboratory (Galbraith Laboratories, Inc., Knoxville, TN). Testing for the presence of sulfide in the [^{11}C]L-leucine solution was done using lead acetate test strips.

RESULTS AND DISCUSSION

HPLC resolution of $[^{11}C]$DL-tryptophan was used to obtain up to 75 mCi of $[^{11}C]$L-tryptophan. Synthesis and resolution required ~ 55 min and gave an overall radiochemical yield of 10-20%. Production yields have not been optimized for $[^{11}C]$L-leucine, but preliminary radiochemical yields were 20-25% and the production time ~ 60 min. No radioactive impurities were detectable for either of the products by HPLC.

The elution profile (Fig. 1) for crude unlabelled DL-leucine reaction mixture spiked with crude ^{11}C-labelled DL-leucine reaction mixture and with

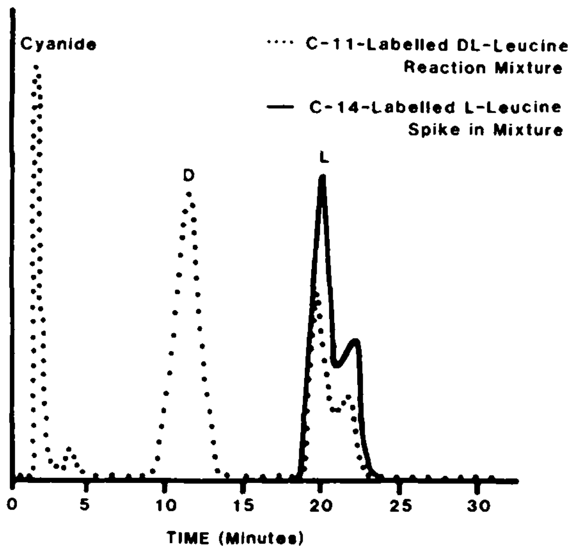

Fig. 1. HPLC elution profile obtained with a mixture of 1.3-1.5 mL of crude unlabelled DL-leucine reaction mixture, 0.5 mL of concentrated HCl, 9.6 mg of cupric acetate, 11.7 mg of L-proline, 5 μCi of $[^{14}C]$L-leucine (New England Nuclear, Boston, MA), and ~ 1 mCi of crude $[^{11}C]$DL-leucine reaction mixture.

^{14}C-labelled L-leucine showed good correlation in elution time for ^{11}C- and ^{14}C-labelled L-leucine. The analogous experiment using a ^{14}C-labelled D-leucine spike (Fig. 2) likewise gave good correlation between ^{11}C- and

^{14}C-labelled D-leucine. Excellent resolution of the D- and L-leucine peaks was observed in both cases. The resolution times for the D- and L-enantiomers were 13 min and 23 min, respectively.

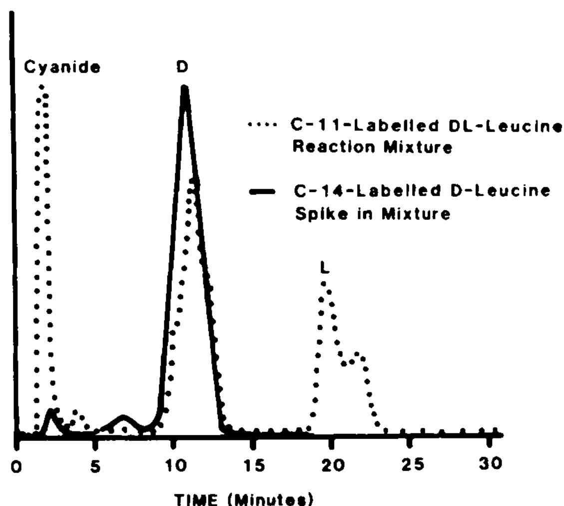

Fig. 2. HPLC elution profile obtained with a mixture of 1.3-1.5 mL of crude unlabelled DL-leucine reaction mixture, 0.5 mL of concentrated HCl, 9.6 mg of cupric acetate, 11.7 mg of L-proline, 5 µCi of [^{14}C]D-leucine (ICN Chemical and Radioisotope Division, Irvine, CA), and ~ 1 mCi of crude [^{11}C]DL-leucine reaction mixture.

The elution pattern (Fig. 3) for crude unlabelled DL-tryptophan reaction mixture that had been spiked with crude ^{11}C-labelled DL-tryptophan and with ^{14}C-labelled L-tryptophan similarly showed good correlation in elution time between the ^{11}C- and ^{14}C-labelled L-tryptophan peaks. The D- and L-tryptophan peaks were well resolved, having elution times of 11 min and 13 min, respectively. Because ^{14}C-labelled D-tryptophan is not commercially available, it was not possible to do the analogous experiment using this material as a spike.

A striking feature of the leucine resolution profiles was the splitting of the L-leucine peak (Figs. 1 and 2). Although much less obvious, this splitting was also apparent for the D-enantiomer in the tryptophan resolution profile (Fig. 3). The reason for this peak splitting is not known.

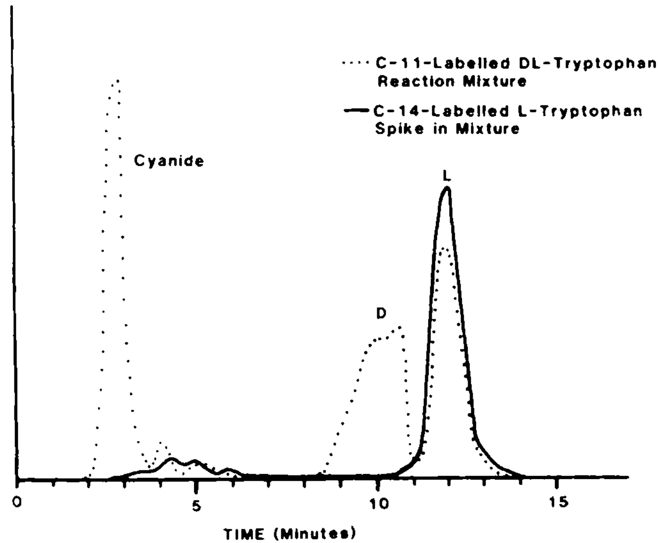

Fig. 3. HPLC elution profile obtained with a mixture of 1.3-1.5 mL of crude unlabelled DL-tryptophan reaction mixture, 0.5 mL of concentrated HCl, 9.6 mg of cupric acetate, 11.7 mg of L-proline, 5 μCi of [^{14}C]L-tryptophan (New England Nuclear), and ~ 1 mCi of crude [^{11}C]DL-tryptophan reaction mixture.

After resolution of [^{11}C]DL-leucine by our HPLC method, it was necessary to break up the L-leucine-copper-L-proline complex by precipitation of the copper with hydrogen sulfide. The resulting cupric sulfide was removed by filtration, and the excess hydrogen sulfide was removed by cation-exchange chromatography. Standard tests for sulfide ions in the purified eluate were negative. For resolution of [^{11}C]DL-leucine the L-proline present in the HPLC mobile phase was not removed, as was also the case for [^{11}C]DL-valine resolution (8). Assuming collection of 20 mL of HPLC eluate, there would be 39 mg of L-proline in an entire batch of [^{11}C]L-leucine. As we have shown previously (8), this level of L-proline is nontoxic and does not affect the biodistribution of a radiolabelled amino acid. In fact, we have studied 30 patients using [^{11}C]L-valine prepared analogously with no evidence of any adverse reaction (K.F. Hübner, Oak Ridge Associated Universities, unpublished data).

On the other hand, in the resolution of [^{11}C]DL-tryptophan, the complex of

L-tryptophan with copper and L-proline is weaker than that of L-leucine, and this complex was decomposed when the acidified HPLC eluate was subjected to cation-exchange chromatography. Use of ^{14}C-labelled L-proline and copper-64 as radiotracers showed that all of the L-proline and copper was removed by the HCl and water washes. The L-tryptophan, however, was tightly bound by the cation-exchange resin until eluted with base. Commercial analysis showed the copper level in a typical preparation to be only 0.9 mg/L or 13.5 μg in the usual 15 mL of eluate.

Of the three methods that have been used for resolution of ^{11}C-labelled amino acid racemates, only the enzymatic method using immobilized amino acid oxidases (1,2) and our HPLC method (8) appear to be generally useful. Gil-av et al. (9) investigated an HPLC method similar to that used in this report and found it to resolve 16 out of the 18 racemic amino acids studied. The activity of immobilized D-amino acid oxidase toward a variety of amino acid substrates is likewise subject to considerable variability (10,11), such that the enzymatic resolution method will likely be very useful for certain amino acids and not at all for others. It would appear that the choice of a preferred resolution method for ^{11}C-labelled amino acid racemates should be made on an individual basis.

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