Production of L-[1-^{11}C]valine by HPLC Resolution

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Based on a recently developed analytical technique, preparative high-performance liquid chromatographic (HPLC) resolution of DL-[1-^{11}C]valine has been achieved. A conventional reverse-phase HPLC column and a chiral mobile phase (aqueous solution of L-proline, cupric acetate, and sodium acetate) were used. The copper can be removed from the L-valine fraction by precipitation as the sulfide, and final purification by cation-exchange chromatography yields L-[1-^{11}C]valine in a form that is acceptable for clinical positron tomographic studies. This purification method does not remove the L-proline introduced in the resolution process, but added L-proline did not affect the tissue distribution of L-[1-^{14}C]valine in rats. We have produced up to 60 mCi of L-[1-^{11}C]valine in an overall synthesis and resolution time of 50 min. This procedure should be adaptable to the rapid resolution of other C-11-labeled amino acid racemates.

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We have previously reported a rapid, high-temperature, high-pressure modification of the Bücherer-Strecker synthesis for the production of C-11-carboxyl-labeled amino acids (*1*,*2*). This method is quite general for C-11-labeled neutral amino acids, and, with appropriate modifications, can also be used for C-11-labeled DL-tryptophan. Currently we are investigating clinically four C-11-carboxyl-labeled amino acids: DL-[1-^{11}C]tryptophan (*3*) and DL-[1-^{11}C]valine (*4*) for pancreatic imaging (*5*), and 1-aminocyclobutane[^{11}C]carboxylic acid (*6*) and 1-aminocyclopentane[^{11}C]carboxylic acid (*7*) for tumor imaging (*8*).

The Bücherer-Strecker synthesis gives racemic mixtures for those amino acids with asymmetric centers, which includes all of the naturally occurring amino acids except glycine. For physiological studies, such as positron tomographic investigations of protein synthesis in the brain and other organs, C-11-labeled L-amino acids would be required.

Digenis and Casey, working in collaboration with our research group, have prepared optically active C-11-labeled D- and L-phenylalanine by using immobilized amino-acid oxidases to destroy the undesired enantiomer selectively (*9*). Wu et al. (*10*) have recently reported the resolution of DL-[1-^{11}C]tryptophan using human serum albumin coupled to a sepharose resin.

Recently, direct resolution of racemic mixtures by column liquid chromatography has been reported (*11*). Either chiral packings made from natural or synthetic optically active polymers or conventional packings with a chiral mobile phase are used. One such method (*12*) uses a reverse-phase high-performance liquid chromatographic (HPLC) column with a chiral mobile phase (L-proline and cupric acetate in aqueous sodium acetate) for resolution of amino acid racemates. We report here the application of this method to the rapid resolution of DL-[1-^{11}C]valine for production of clinically useful amounts of L-[1-^{11}C]valine.

MATERIALS AND METHODS

Our HPLC system* was fitted with a 2.0-ml sample loop and a preparative column (10 mm i.d. × 25 cm long, Ultrasphere ODS, 5-μ†). The column was equilibrated with a mobile phase consisting of 0.017 *M* L-proline and 0.008 *M* cupric acetate in 0.03 *M* aqueous sodium ace-

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tate, which had been previously degassed under vacuum. The flow rate was adjusted to 7.2 ml/min, giving a pressure of 3600 psi.

Crude C-11-labeled DL-valine was prepared at the Oak Ridge National Laboratory's 86-in. cyclotron complex as previously described (*2*), except that the following measures were taken to minimize the volume of the reaction mixture: (a) the gas-absorption column was not rinsed with water and (b) 0.5 ml of 12 *N* NaOH was used in place of 1.0 ml of 6.25 *N* NaOH for hydrolysis of the hydantoin. The crude C-11-labeled DL-valine reaction mixture (~1.2 ml) was filtered, adjusted to a pH of 5–6 using concentrated HCl, and combined with 9.6 mg of cupric acetate and 11.7 mg of L-proline in 0.1 ml of water. The mixture was passed through a 0.22-μ microfilter, and the filtrate then loaded onto the HPLC column. Elution of the C-11-labeled mixture was monitored with a shielded gamma probe. The fraction containing the L-valine (~20 ml) was acidified by adding 2 ml of 6 *N* HCl, and 3 ml of a saturated aqueous solution of hydrogen sulfide (made fresh that day) was added to precipitate the copper as cupric sulfide. The mixture was filtered and the filtrate loaded onto a 1.0 × 15 cm, AG 50W-X2, 50–100-mesh cation-exchange bed in the hydrogen form† (previously washed with water and 1 *N* HCl). After loading, the column was washed twice with water to remove hydrogen sulfide and other impurities, and L-[1-^{11}C]valine was eluted with 0.2 *N* NaOH. The elution was monitored using a shielded gamma probe to minimize the volume of eluate (~15 ml). Final radiopharmaceutical processing (neutralization, microfiltration, and pyrogen testing) was carried out as previously described (*2*).

In the development of the resolution method, experiments were carried out using tracer amounts of C-11 and C-14 activity under conditions that would simulate the resolution of a full batch of crude DL-[1-^{11}C]valine. The crude unlabeled DL-valine reaction mixture (1.5 ml) (*2*) was spiked with 20 μl (~1 mCi) of a crude C-11-labeled DL-valine reaction mixture and with 50 μl (5 μCi, 0.01 mg) of L-[1-^{14}C]valine.‡ This mixture was combined with 0.5 ml of concentrated HCl, 3.2 mg of cupric acetate, and 3.9 mg of L-proline, microfiltered, and loaded onto the HPLC column. Elution of the C-11 activity was followed using a custom monitor system with a liquid-column single-channel analyzer.‖ Carbon-14 assays were made by collecting fractions using a drop-counting fraction collector [30 drops (2.2 ml) per fraction], followed, after decay of the C-11, by liquid scintillation counting of aliquots of each fraction.

The radiochemical purity of the C-11-labeled L-valine produced by HPLC resolution was determined using an analytical column (4.6 mm i.d. × 25 cm long, Spherisorb ODS, 5-μ§). (Spherisorb ODS, 5 μ and Ultrasphere ODS, 5 μ are similar in their ability to resolve DL-[1-^{11}C]valine, and can apparently be used interchangeably.) Twenty microliters of purified, neutralized L-[1-^{11}C]-valine was loaded onto the column (using a 20-μl sample loop). The flow rate was 2.0 ml/min, and elution of radioactivity was again followed using a liquid-column single-channel analyzer monitor system.

Because the L-[1-^{11}C]valine produced by this method contains L-proline (39 mg, assuming collection of 20 ml of HPLC eluate), the effect of added L-proline on the tissue distribution of C-14-labeled L-valine was tested in three-month-old Buffalo rats¶ (five animals per group). The rats were injected by tail vein with L-[1-^{11}C]valine (5 μCi = 0.01 mg L-valine/kg body weight), with or without 1 mg of added L-proline per kg of body weight. At 30 min after injection, the animals were killed by exsanguination under light ether anesthesia. Tissue samples were weighed, dissolved in NCS tissue solubilizer,** and assayed by liquid-scintillation counting.

For radiation dosimetry calculations, three-month-old male and female Fischer rats (five animals per group) were injected by tail vein with L-[1-^{14}C]valine (5 μCi = 0.01 mg L-valine/kg body weight). The animals were killed at 30 min after injection, and the tissue samples were analyzed as above.

RESULTS AND DISCUSSION

HPLC resolution of DL-[1-^{11}C]valine was used to obtain up to 60 mCi of L-[1-^{11}C]valine with a specific activity of approximately 2 Ci/mmole. The radiochemical yield for the two-step Bücherer-Strecker synthesis with HPLC resolution was 15–20%, and the entire process required ~50 min.

The elution pattern (Fig. 1) for the crude unlabeled DL-valine reaction mixture that had been spiked with crude C-11-labeled DL-valine reaction mixture and with C-14-labeled L-valine showed an early peak due to unreacted cyanide along with almost completely resolved D- and L-valine peaks. A minor peak between the cyanide and D-valine peaks was apparently due to unhydrolyzed hydantoin. We have not discovered the reason for the apparent splitting of the D-valine peak, in contrast with the sharpness of the L-valine peak, and for a shoulder on the front edge of the L-valine peak, seen with both C-11- and C-14-labeled L-valine. Good correlation in elution time was obtained for C-11- and C-14-labeled L-valine.

A single radioactive peak corresponding in elution time to L-valine was obtained when HPLC-resolved L-[1-^{11}C]valine was assayed by analytical HPLC. This shows that HPLC resolution of DL-[1-^{11}C]valine yields radiochemically pure L-[1-^{11}C]valine.

The optical resolution of DL-valine is based on the separation by HPLC of the diastereoisomeric complexes, L-proline-copper-L-valine and L-proline-copper-D-valine. Gil-av and co-workers (*12*) found this method useful for resolution of a total of 16 racemic amino acids in addition

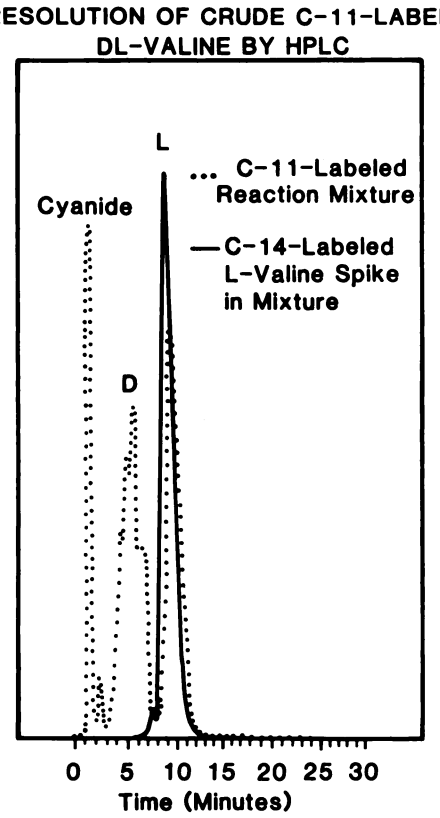

FIG. 1. HPLC elution profile obtained with a mixture of 1.5 ml of crude unlabeled DL-valine reaction mixture, 0.5 ml of concentrated HCl, 3.2 mg of cupric acetate, 3.9 mg of L-proline, 50 μl (5 μCi) of L-[1-^{14}C]valine, and 20 μl of crude C-11-labeled DL-valine reaction mixture.

to valine, examples of which are alanine, leucine, isoleucine, methionine, and tyrosine. Separation factors (the ratio of the corrected retention times of the two enantiomers) of up to 4.8 were obtained. The only amino acids studied that were not resolved by the method were aspartic acid and threonine. We are currently studying the applicability of our preparative adaptation of this technique, which was used for resolution of DL-[1-^{11}C]valine, to the resolution of other C-11-labeled amino acids of interest in nuclear medicine.

Our HPLC method offers certain advantages over the other methods that have been used for resolution of C-11-labeled amino acid racemates. It is less time consuming and requires fewer manipulations than the enzymatic method using immobilized amino-acid oxidase (*9*), which involves preliminary ion-exchange purification of the amino-acid reaction mixture. The human serum albumin–sepharose method (*10*) is apparently applicable only to resolution of C-11-labeled DL-tryptophan (*13*).

The only potential disadvantage to our method that is apparent is that the L-proline present in the HPLC mobile phase is not removed. However, this should not prevent the clinical usefulness of L-[1-^{11}C]valine, since no effect on the tissue distribution of L-[1-^{14}C]valine was

TABLE 1. EFFECT OF ADDED L-PROLINE ON THE TISSUE DISTRIBUTION OF L-[1-^{14}C]-VALINE IN THE RAT AT 30 MIN AFTER INJECTION

	Percent administered dose/g	
Tissue	L-[1-^{14}C] valine (0.01 mg/kg)	L[1-^{14}C]valine (0.01 mg/kg) + L-Proline (1 mg/kg)
Pancreas	10.87± 0.47*	11.10 ± 0.31
Liver	1.11 ± 0.05	1.20 ± 0.02
Spleen	0.84 ± 0.07	0.82 ± 0.03
Kidney	0.75 ± 0.05	0.76 ± 0.03
Lung	0.58 ± 0.08	0.52 ± 0.03
Muscle	0.23 ± 0.02	0.23 ± 0.01
Marrow	1.45 ± 0.04	1.42 ± 0.04
Blood	0.16 ± 0.01	0.16 ± 0.01
Small intestine	1.77 ± 0.09	1.72 ± 0.04
Urine	0.28 ± 0.06	0.23 ± 0.03
Brain	0.12 ± 0.01	0.11 ± 0.00

* Mean of five animals ± s.e.; normalized to body weight of 250 g.

observed when L-proline was coadministered in the amounts that would result from the use of our HPLC resolution procedure (Table 1). Processing of 20 ml of HPLC eluate would lead to 39 mg of L-proline in an entire batch of L-[1-^{11}C]valine. Assuming that blood comprises 7% of the total human body weight and has a hematocrit of 40, there would be an average of 69.4 mg of L-proline in the blood volume of a 70-kg person (*14*). Administration of 39 mg of L-proline to a 70-kg person would give a level of 0.56 mg/kg. In addition, the LD_{50} toxicity value for intraperitoneally administered L-proline in mice is reported to be 8.06 g/kg (*15*). Thus, in administering our preparation to humans, we would be below the mouse LD_{50} value with a safety factor of 14,400.

Our method does, however, effectively remove both copper and sulfide ions. The copper level in the purified L-[1-^{11}C]valine solution was below the sensitivity of the ferric thiocyanate spot test (*16*), i.e., <0.4 μg Cu/ml (<6 μg Cu total). Testing for sulfide by means of lead acetate test strips was negative.

Chemical toxicity from L-valine would also be negligible. An entire batch of L-[1-^{11}C]valine would be expected to contain no more than 4 mg of L-valine, giving 0.06 mg/kg for a 70-kg person. L-valine is present to the extent of 2.4 (2.0–2.9) mg per 100 ml of normal human blood (*14*). Using the same assumptions as stated above for L-proline, there would be ~118 mg of L-valine in the blood volume of a normal 70-kg human. Thus the administration of the carbon-11 preparation would only increase the blood pool of L-valine by ~3%.

TABLE 2. ESTIMATED RADIATION DOSE FROM L[1-^{11}C]VALINE TO VARIOUS ORGANS OF MAN*

Tissue	Radiation dose (rads/mCi)
Total body	0.011
Pancreas	0.19
Liver	0.028
Spleen	0.022
Kidney	0.019
Bone marrow	0.021
Ovary	0.011
Testis	0.0073

* Based on 30-min tissue distribution in male and female rats following intravenous administration of L-[1-^{14}C]valine (0.01 mg/kg) assuming immediate uptake and no further change in distribution.

Because of the 20.4-min half-life of C-11, the radiation dose to patients from L-[1-^{11}C]valine will also be reasonably low (Table 2). The critical organ, the pancreas, would receive 0.19 rads/mCi, or 3.8 rads for a 20-mCi dose.

Positron tomographic studies with L-[1-^{11}C]valine are potentially useful both for pancreatic imaging and for measuring protein synthesis in the brain. Pancreatic carcinomas and other abdominal tumors have generally shown unexpectedly high uptakes of DL-[1-^{11}C] tryptophan and DL-[1-^{11}C]valine (*5*). This may result from the selective uptake of the D-amino acid enantiomer by the tumor, as observed by Tamemasa and co-workers (*17*). On the other hand, L-[1-^{11}C]valine would not suffer from this disadvantage. Pancreatic carcinomas would be expected to appear as defects in the normal pancreatic uptake of L-[1-^{11}C]valine, as is the case with L-[^{75}Se]selenomethionine (*18*) and L-[methyl-^{11}C]-methionine (*19*). In addition, the greater pancreatic uptake of L-[1-^{11}C]valine [10.87 %/g versus 6.47 %/g for the racemic mixture (*4*)] should lead to improved images of the pancreas and permit use of smaller tracer doses. The uptakes by liver, small intestine, and bone marrow are also higher for the L- form, reflecting its greater utilization in protein synthesis, whereas the blood concentration is lower, suggesting longer retention of the D-enantiomer in the blood. Positron tomographic assessment of L-[1-^{11}C]valine for pancreatic imaging is currently in progress at our institution. A collaborative study with the University of Michigan is also utilizing L-[1-^{11}C]valine for measuring regional brain protein synthesis (*20*) in patients with various neurological diseases. Although the uptake of L-[1-^{14}C]valine in the rat brain at 30 min is only 0.12%/g (Table 1), we have been able to obtain useful positron tomographic brain data in humans with doses of 10–15 mCi of L-[1-^{11}C]-valine (K. F. Hübner, Oak Ridge Associated Universities, unpublished data).

FOOTNOTES

* 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.

† Altex Scientific, Inc., Berkeley, CA.

‡ New England Nuclear Corp., Boston, MA.

‖ Packard Instrument Co., Downers Grove, IL.

§ Laboratory Data Control, Riviera Beach, FL.

¶ Laboratory Supply Company, Indianapolis, IN.

** Amersham/Searle, Arlington Heights, IL.

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