REVISTA ESPAÑOLA DE FISIOLOGIA, 26, 321-324. 1970

Enzymic Obtaining of U-14C 4-Aminobutyric Acid From U-14C Glutamic Acid

M. Cascales, J. M. Ortiz and A. Santos-Ruiz

Department of Biochemistry. Faculty of Pharmacy.
University of Madrid (Spain)

(Received on 3 June, 1970)

M. CASCALES, J. M. ORTIZ and A. SANTOS-RUIZ. *Enzymic Obtaining of U-14C 4-Aminobutyric Acid from U-14C Glutamic Acid.* R. esp. Fisiol., 26, 321-324, 1970.

Uniformly labelled 14C 4-aminobutyrate has been obtained from U-14C glutamic acid by means of enzymic decarboxylation. U-14C 4-aminobutyrate obtained was isolated following the techniques of aminoacid resolution by paper chromatography. After elution, the product obtained showed 20 % of the radioactivity of the initial U-14C glutamate.

In the face of the difficulty of obtaining a commercial 14C 4-aminobutyrate uniformly labelled, and of the necessity of this product to continue our works in progress (2), we decided to test the possibility of obtaining it from uniformly labelled 14C glutamic acid, by means of enzymic decarboxylation. The enzyme which catalyzes this reaction, glutamate decarboxylase, was obtained from *Lupinus albus* seeds.

Materials and Methods

The U-14C glutamic acid (0.1 mc/mol) was supplied by Amershan (England) and through the «Junta de Energía Nuclear» in Madrid. Pyridoxal-5-phosphate, coenzyme in the reaction, proceeded from Roche. All the others reagents used in the preparation of buffers, chromatographic solvents, etc., were suplied by Merck, Light and Analema. Glutamate decarboxylase was purified following the method used by MAYOR (1).

The concentration of the products on the reaction flask was as follows: sodium phoshpate buffer pH 5.7, 55 μmoles; pyridoxal-5-phosphate 0.1 μmol; U-14C sodium glutamate pH 5.7, 9 μmoles and 45 μC. Purified enzyme was added in the concentration of 2.2 mg. The final total volume was 1.1 ml.

A glass side tube containing 0.5 ml of 5M KOH was adapted to the outlet of the reaction flask to collect the 14C CO_2 released in the reaction as a radioactive

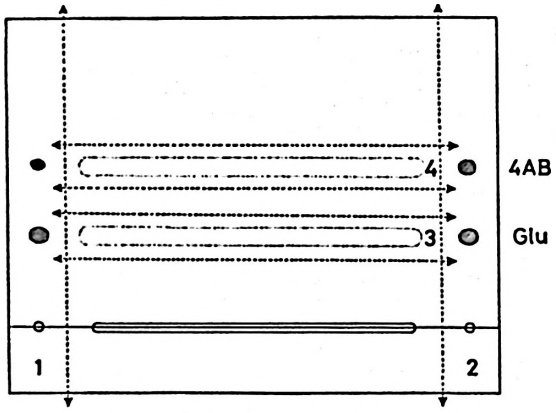

Fig. 1. *Paper chromatography separation of the U-14C glutamate and U-14C 4-aminobutyrate.*

The enzymic obtaining of U-14C 4-aminobutyrate was as follows: A mixture of, sodium phosphate buffer pH 5.7, 55 μmoles; pyridoxal-5-phosphate 0.1 μmol; sodium U-14C glutamate 9 μmoles and 45 μC and purified glutamate decarboxylase 2.2 mg in a final volume of 1.1 ml, was incubated during 60 minutes at 37°. After boiling and centrifuging, the supernatant fluid was resolved by paper chromatography, as is shown in the figure. When the chromatogram was developed and dried, 1 and 2 strips were cut out following dashed lines. 1. Strip corresponding to an aliquot of the radioactive supernatant fluid (see figure 2). 2. Strip corresponding to a non radioactive standard sample with glutamate and 4-aminobutyrate. Both 1 and 2 strips were stained with ninhidrin to allow delimiting the place where U-14C glutamate and 4-aminobutyrate appeared. After that other two strips were cut out: 3. Strip corresponding to U-14C glutamate non transformed, and 4. Strip corresponding to U-14C 4-aminobutyrate obtained. 3 and 4 were eluted separadtly with distilled water.

carbonate. When the reagents were all mixed together, the mixture was incubated in a water bath at 37° for 60 minutes, with gently shaken. The enzymic reaction was then stopped by immersing the reaction flask in a boiling water bath for 10 minutes. The precipitated protein were removed by centrifuging at 6000 g. In the supernatant fluid, both substrate and product of the reaction were resolved by paper chromatography on Whatman 1, using as solvent butanol/acetic acid/water (4:1:5). Once the chromatogram were developped and dried, the place where the 4-aminobutyrate appeared was delimited by standard samples. The strip of paper corresponding to the area of the U-14C 4-aminobutyrate was cut out (Fig. 1) and was eluted with distilled water by summerging one end of the strip, held between two glass plates, into a recipient holding distilled water. The water runs up the strip of paper and the runs down it again, bringing the U-14C 4-aminobutyrate to be eluted with it. In the first 3 ml, caught in a small tube placed at the other end of the strip, was collected almost all the product obtained.

The strip of the chromatogram containing the resolved sample, was passed through a Nuclear Chicago 1002 gas flow counter with a recording ratemeter (Actigraph III). The graph obtained (Fig. 2) showed the total separation and the efficiency of the reaction. The efficiency was checked again by measuring the radioactivity of the initial glutamate and of the 4-aminobutyrate obtained aliquots in the counting planchets in a Tracerlab mod. SC-33 ANT «1000» counter. The efficiency of the reaction according to the calculations corresponding to the measurements of the areas of the radiochromatograms, was 20 %. Later analysis of the radioactivity of the eluted product in the counting planchets, confirmed these results.

Results and Discussion

In the enzymic decarboxylation of the U-14C glutamic acid, there is a loss of the radioactivity per molecule of 20 %, in the form of 14C CO_2. Due to this, the radiactivity of the 4-aminobutyrate obtained shall be the 80 % per mol of radioactive glutamate transformed. The efficiency of

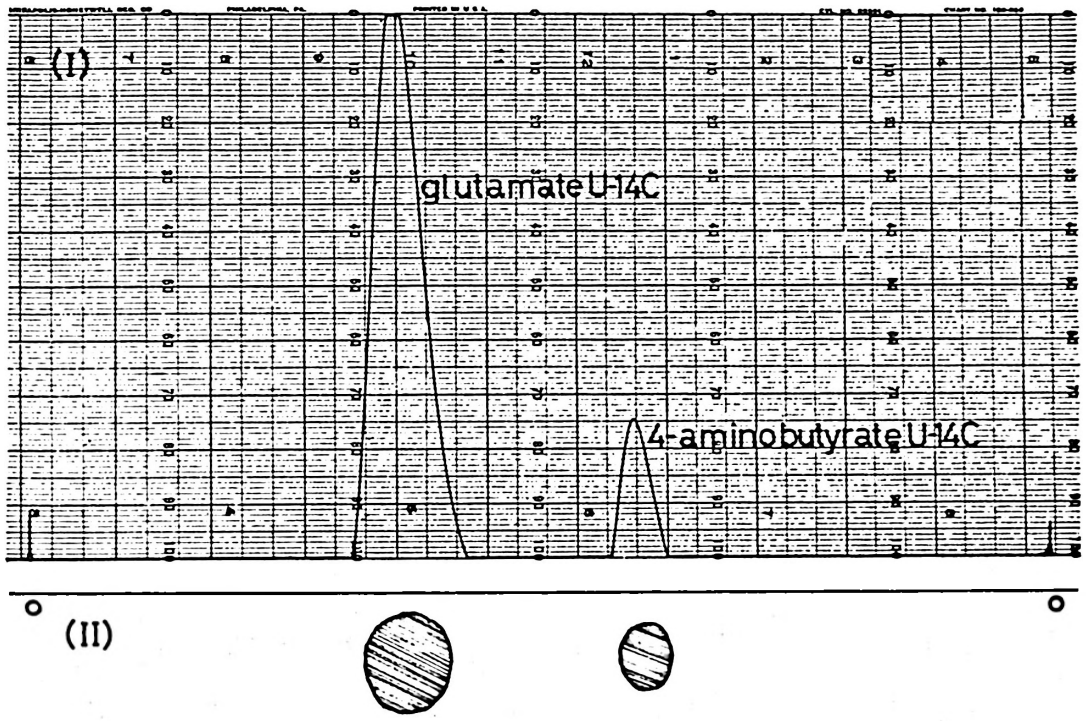

Fig. 2. *Test to check chromatographic resolution of U-14C glutamic acid and U-14C 4-aminobutyric acid.*

(I) Chart graph corresponding to 14C radioactivity of glutamate and 4-aminobutyrate both uniformly labelled, recorded in a Nuclear Chicago 1002 gas flow counter. The counting conditions were as follows: high voltage 960, colimator 3, scan speed 30 cm/hour, scale 100 K c/m and time constant 50. The efficiency of the reaction was 20 %. Remainded U-14C glutamate 75 %, obtained U-14C 4-aminobutyrate 20 % and released 14C CO_2, fixed as 14C CO_3K_2, 5 %. (II) Paper chromatogram strip (see figure 1) showing the spots corresponding to both substrate and product of the enzymic reaction after ninhidrin treatment. The strip was passed through Actigraph III, given the results as a chart graph (I) above.

the enzymic decarboxylation of glutamic acid cannot be greater than 25 %, since the 4 aminobutyrate, the product in the reaction, acts as an inhibiting factor in the same reaction. As the U-14C initial glutamate was in concentration of 1 μC per ml, the product obtained after elution was then diluted so that the isotopic dilution reached approximately 0.8 μC per ml. 0,1 ml aliquots of U-14C standard (1 μC/ml) and of the product obtained, were checked for radioactivity, and they gave the following results: U-14C glutamate (0.1 μC/ml), 57,430 c.p.m. and U-14C 4-aminobutyrate (0.8 μC/ml approx.), 46,068 c.p.m. If the theoretical calculation should give for the U-14C 4-aminobutyrate 45,940 c.p.m., in the same conditions, the agreement found between the theoretical and experimental values is manifest and shows the efficiency and effectiveness of our method.

The non transformed U-14C glutamate, 75 % of the initial value, can be recuperated also by elution, and under the same conditions used for the U-14C 4-aminobutyrate (Fig. 1). This allows to use it for further experiments, once the inhibi-

ting product from the decarboxylizing reaction was removed.

Acknowledgments. We wish to thank Dr. P. GONZÁLEZ for isolating glutamate decarboxylase.

References

1. MAYOR, F.: *Anal. Real Acad. Farm.*, 25, 219, 1959.
2. ORTIZ, J. M.: Doctoral Thesis. University of Madrid. 1969.