ONE-POT SYNTHESIS OF 85 % ENRICHED $^{13}C(U)$-L-ASPARAGINE FROM $^{13}C(U)$-L-ASPARTIC ACID

Hung LAM-THANH, René MERMET-BOUVIER, Serge FERMANDJIAN and Pierre FROMAGEOT

Service de Biochimie, Département de Biologie, CEN-SACLAY
91191 - GIF-sur-YVETTE Cedex, FRANCE

SUMMARY

An improved high yield, racemization free synthesis of 85 % $^{13}C(U)$-L-Asparagine by β- carboxamide formation on $^{13}C(U)$ enriched L-Aspartic acid is reported.

Key Words : $^{13}C(U)$L-Asparagine - β-Carboxamide

INTRODUCTION

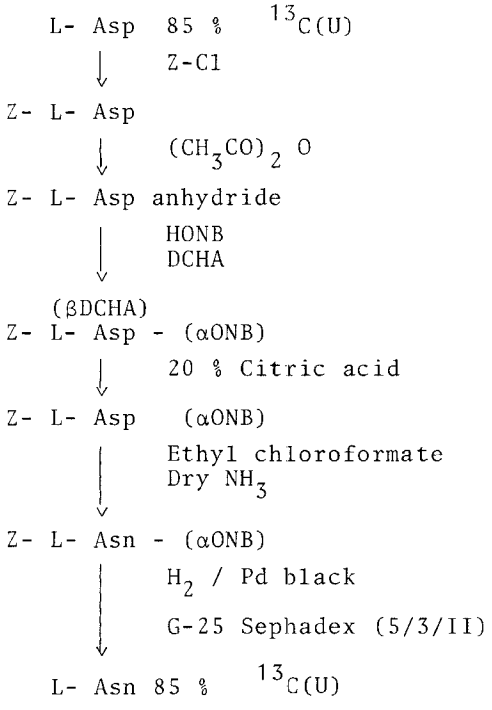
As incorporation of amino acids labeled with stable isotopes (^{13}C, ^{15}N, ^{2}H) into peptides (1) (2) or proteins (3) becomes widely used for spectroscopic purposes applied to biology (conformation - activity relationship) and medicine (pharmacokinetics,....), the preparation of ^{13}C-enriched-L-asparagine as a precursor for metabolism studies or as starting material for peptide synthesis, seemed interesting. We report here an improved high yield synthesis of $^{13}C(U)$ labeled L-asparagine starting with $^{13}C(U)$ L-aspartic acid which is a biosynthetical product obtained in our Laboratory (4).

0362-4803/83/010143-06$01.00

Received June 28, 1982

RESULTS AND DISCUSSION

The preparation of L-asparagine labeled with ^{15}N from L-aspartic acid has been described by Yamamoto in 1963 (5). He used L-aspartic acid anhydride which was then esterified specifically on the α-carboxylic group with benzyl alcohol (5). In our case TLC analysis has shown that the esterification led to the concomittant formation of β-benzyl and α-β-dibenzyl ester of aspartic acid instead of the single N- and Cα-protected L-aspartic acid useful for further chemical transformation on the Cβ-carboxylic group. We used then the Cordopatis method (6) for the preparation of N-Trityl-α-benzyl -L-aspartic acid. This however probably because only few chemical details were available in the report (6) led only to a partially pure compound. Alcaline hydrolysis of N-Trityl-L-aspartic acid (α,β) dibenzyl ester was probably the critical step. Therefore the one-pot synthesis or straight-on synthesis of $^{13}C(U)$-L-asparagine from $^{13}C(U)$ -L-aspartic acid was carried out according to scheme I.



SCHEME 1

Outline of synthesis scheme for 85 % enriched $^{13}C(U)$ - L-asparagine from $^{13}C(U)$ - L-aspartic acid.

The straight-on synthesis of L-asparagine was achieved without purification of the intermediate and seems an advantageous method for two reasons :

- it is not time consuming
- the yield is good (calculated from L-aspartic acid) : 40 - 60 %

Regarding Yamamoto's method, β-carboxamide reaction was performed with the mixed anhydride method instead of the acyl chloride methods (5). Smooth cleavage of the protecting group of L-asparagine by hydrogenolysis gave no detectable nitrile formation via deshydratation of the β-carboxamide group according to amino acid analysis (7) (Figure 1). Purification of 85 % $^{13}C(U)$-L-asparagine by partition chromatography on G-25 Sephadex is revealed as a suitable method for obtaining pure L-asparagine : this is based on the relative hydrophobicity of the by-product (para-Nitrobenzyl alcohol) with respect to L-asparagine.

EXPERIMENTAL

85 % $^{13}C(U)$ - L - Aspartic acid :

^{13}C -labelling of L-aspartic acid was obtained in large scale from blue-green "Synechococcus" as described (4). Algae were grown in the presence of highly enriched $NaH\ ^{13}CO_3$. Proteins extracted with trichloroacetic acid were hydrolyzed by pronase and then by H_2SO_4 (6N) at 100°C. $^{13}C(U)$-L-aspartic acid was finally separated by ion-exchange chromatography. The enriched amino acid was then collected, evaporated and precipitated with acetone.

85 % $^{13}C(U)$ - L-Asparagine :

2 600 mg (20 mmol) of 85 % $^{13}C(U)$-L-aspartic acid was acylated with benzyl-chloroformate (25 mmol) according to Zervas procedure described by Yamamoto (5). After extraction, 5 300 mg of Z-L-aspartic acid was recovered. The formation of Z-L-aspartic acid anhydride was performed with freshly distilled acetic anhydride (5) : 5 000 mg of Z-L-aspartic acid anhydride was obtained after drying overnight on P_2O_5.

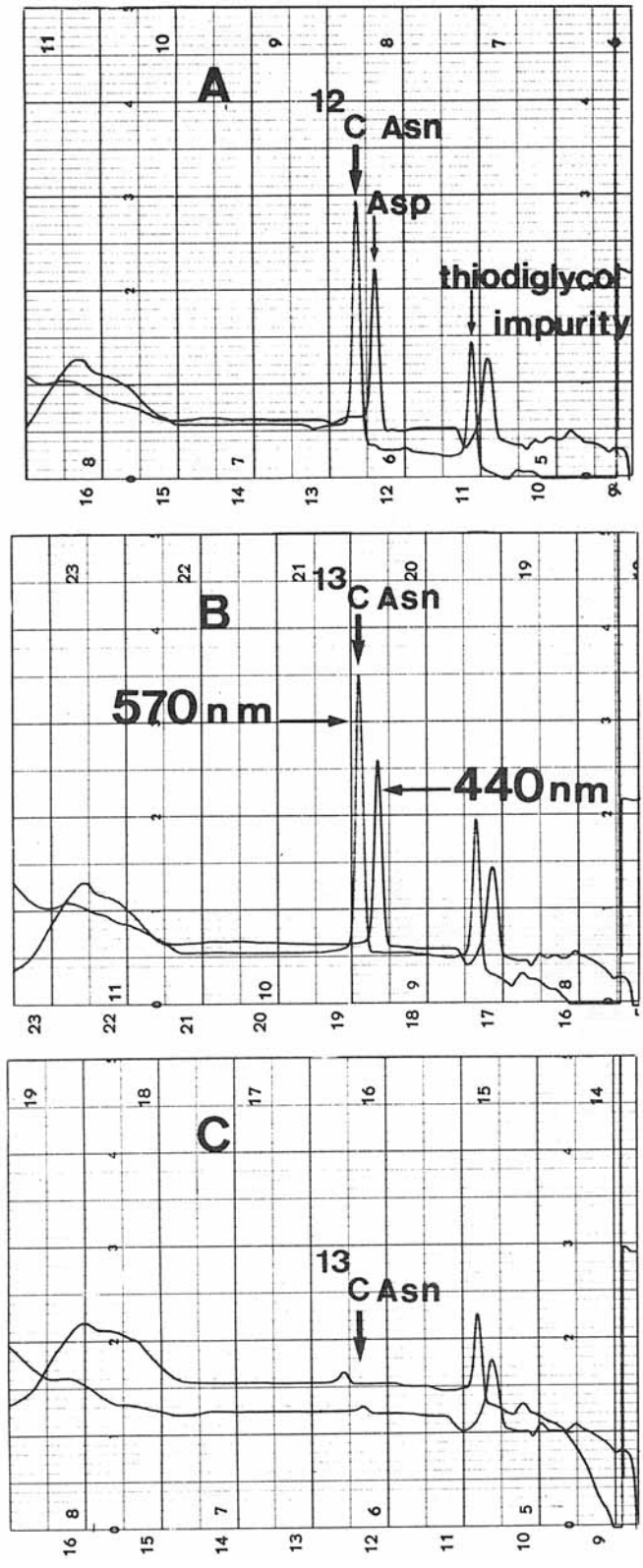


Figure I : Amino acid analysis of 85 % $^{13}C(U)$ -L-Asparagine on an auto amino-acid analyzer LKB, type 4 400 :

A- ^{12}C-L-Asparagine, 5 nmol (first nihydrin-positive peak is assigned to thiodiglycol impurity)

B- ^{13}C-L-Asparagine

C- ^{13}C-L-Asparagine after L-amino-oxydase digestion.

Dicyclohexylammonium salt of benzyloxycarbonyl L-aspartic acid α - p - nitrobenzyl ester was prepared according to Schröder's procedure (8) and gave 6 500 mg of derivative after washing with 20 % citric acid. Mixed anhydride was performed as described : 6 500 mg of Z-L-aspartic acid- α-p-nitrobenzyl ester was dissolved in 100 ml of absolute tetrahydrofurane (peroxyde free), neutralized with 2 ml (20 mmol) of N-methyl-morpholine and cooled to -20°C. Ethylchloroformate was then added in four fractions of 1.8 ml (20 mmol). After 30 min at -20°C, dry ammonia (NH_3 through KOH pellets) was bubbled in the medium with a slow stream during 30 min at -20°C, then 2 hours at 0°C. The solvent was evaporated to dryness and the residue was taken up in CH_2Cl_2 (300 ml). This solution was washed with 3 x 100 ml of 5 % $NaHCO_3$, then with water and dried ($MgSO_4$). After removal of the solvent, the crude product was dissolved in 100 ml of methanol and 10 ml of acetic acid, mixed with 500 mg of palladium black (10 % Pd) and submitted for overnight hydrogenolysis. The product obtained after removal of catalyst and solvent was then chromatographied on G-25 Sephadex fine column (80 cm x 2.5 cm). Elution with 500 ml of the organic phase of I-Butanol/Pyridine/Acetic acid 0.1 % v/v : 5/3/II gave fractions containing coloured products (e.g. p-nitrobenzyl alcohol). ^{13}C-L-Asparagine emerged with the elution by the aqueous phase of the same solvent. Fractions containing ^{13}C-L-Asparagine according to TLC analysis were collected, evaporated and lyophilized (yield : 1650 mg or 60 %).

Characterization of 85 % ^{13}C (U)-L-Asparagine was performed by three methods :

1 - amino acid analysis : ^{13}C-labeled L-Asparagine has the same retention time as ^{12}C-L-Asparagine (Figure I-A and B).

2 - elemental analysis : found (C : 37.12, O : 35.25, N : 20.49, H : 6.10) calculated (C : 37.96, O : 35.45, N : 20.67, H : 5.90)

3 - racemization test : degradation of 85 % ^{13}C(U)-L-Asparagine by L-amino oxydase according to the Crotalus adamenteus L-amino oxydase procedure (9) has shown that no detectable racemization occurs during the chemical transformation of ^{13}C-L-Aspartic acid (Figure I-C).

REFERENCES

/1/ LAM-THANH,H., TOMA F., FERMANDJIAN,S., FROMAGEOT,P. and LOFFET,A.
in Proc. 14th. Europ.Peptide Symposium Wepion, Belgium, April 1976, p.609, Editions de l'Université de Bruxelles, LOFFET, A., Bruxelles

/2/ TOMA F., FERMANDJIAN, S., LÖW, M. and KISFALUDY, L., Biopolymers, 20 , 901 (1981)

/3/ CIPOLLO, K.L., LEWIS, C.A. Jr., ELLIS, P.D. and DUNLAP, R.B. J. Biol.Chem. 257, 4398 (1982)

/4/ TRAN-DINH, S., FERMANDJIAN, S., SALA, E., MERMET-BOUVIER, R., COHEN, M. and FROMAGEOT, P., J.Amer.Chem.Soc, 96, 1484 (1974)

/5/ YAMAMOTO, Y., Biochem. Prep. vol.10, p.10, John Wiley and Sons (1963)

/6/ CORDOPATIS, P. and THEODROPOULOS, D., Experientia, 31, 1255 (1975)

/7/ HEMMASI, B. and BAYER, E., Hoppe Seyler's Z. Physio.Chem. 355, 481 (1974)

/8/ SCHRÖDER, E. and KLIEGER, E., Liebigs Ann.Chem. 673, 208 (1964)

/9/ GREENSTEIN, J.P., WINITZ, M. in Chemistry of Amino Acids, vol.2, p.1789, Wiley, New-York (1961)

The nomenclature is in accord with the IUPAC-IUB rules on Biochemical Nomenclature, Biochem.J. 126, 773 (1972). The abbreviations are : Z, Benzyloxycarbonyl, Z-Cl, benzylchloroformate, HONB, p-Nitrobenzyl alcohol, DCHA, dicyclohexylamine, TLC, thin layer chromatography, Trityl, triphenyl-methyl. TLC silica gel plates were developped in 3 solvent systems :

I-Butanol/Acetic acid/Water : 4/I/I)
Chloroform/Methanol/Acetic acid : 95/5/3) for N and C-protected
Hexane/Ethyl acetate/Acetic acid : 20/10/I) amino-acid.

and in 2 other solvent systems :

I-Butanol/Pyridine/Acetic acid 0.1 % : 5/3/11) for amino-acid
I-Butanol/Pyridine/Acetic acid/Water :15/3/10/12)