INCORPORATION OF ISOTOPIC OXYGEN INTO THE CARBONYL GROUP OF ESTERS AND AMIDES

Lawrence C. Kuo, Timothy R. Koch, and Marvin W. Makinen*

Department of Biophysics and Theoretical Biology
The University of Chicago, Cummings Life Science Center
920 E. 58th Street, Chicago, Illinois 60637

SUMMARY

Syntheses and analyses are described for incorporation of ^{17}O from ^{17}O-enriched water into the carbonyl oxygen position of the ester and amide bonds in O-(trans-p-chlorocinnamoyl)-L-β-phenyllactate and N-(benzoyl)-glycyl-L-phenylalanine.

KEY WORDS: O-(trans-p-chlorocinnamoyl-1-^{17}O)-L-β-phenyllactate; N-(benzoyl)-(glycyl-1-^{17}O)-L-phenylalanine.

INTRODUCTION

The detection of ^{17}O in magnetic resonance studies of natural abundance materials is plagued by stringent requirements of high concentrations and extensive signal averaging. This circumstance has been a significant handicap in natural abundance ^{17}O nuclear magnetic resonance studies of esters (1) and peptides (2). Therefore, a means for high enrichment of esters and amides with isotopic oxygen would provide a useful probe of structure in magnetic resonance studies. In recent electron paramagnetic resonance studies, we have employed a synthetic ester substrate in which the carbonyl group of the scissile bond was specifically enriched with ^{17}O to determine the coordination environment of the active site metal ion in the acylenzyme reaction intermediate of carboxypeptidase A (3). We outline the synthesis procedures for selective incorporation of ^{17}O into the carbonyl group of esters and amides developed in the course of these studies.

*To whom to address correspondence.
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Although the procedures described are specific for synthetic substrates of carboxypeptidase A, they can be readily adapted to a variety of esters and peptides.

EXPERIMENTAL

Benzene, tetrahydrofuran (THF), and triethylamine (TEA) were dried over sodium and distilled in glass immediately prior to use. All chemicals in the solid state were dried over P_2O_5 before use. Vacuum evaporations were carried out with an oil pump system connected to a Buchli rotary evaporator. Unlabeled and labeled compounds were analyzed by elemental analysis, mass spectrometry, ultraviolet spectroscopy, and melting point. Unlabeled compounds were prepared by the same synthetic pathway using identical quantities to those described below and shown to correspond identically to those synthesized by published methods. Dicyclohexylcarbodiimide was obtained from Sigma Chemical Company (St. Louis, MO.) and used without further purification. Para-chlorocinnamic acid (Aldrich Chemical Co., Milwaukee, WI.) and N-benzoyl-glycine (Sigma Chemical Co., St. Louis, MO.) were recrystallized from acidified water and dried over P_2O_5 before use. All other chemicals were of the highest purity commercially available.

O-(trans-p-chlorocinnamoyl-1-^{17}O)-L-β-phenyllactate. ^{17}O-enriched water (0.3 ml) (52.79% ^{17}O, 41.79% ^{18}O; Monsanto Research Corporation, Miamisburg, OH.) was added to the acid chloride of p-chlorocinnamic acid (4), (0.4 g, 1.99 mmoles) suspended in 20 ml benzene and stirred for 4 days at ambient temperature in a closed vessel. Upon evaporation of the solvent and drying over P_2O_5, white crystals (0.35 g, 1.92 mmoles, 96% yield) were recovered and then reconverted to the acid chloride (0.18 g, 0.90 mmoles, 46% yield). The acid chloride was condensed with L-β-phenyllactic acid, and the ester product was recrystallized from benzene-hexane (0.089 g, 0.27 mmoles, 27% yield; m.p. 124-125°C).

The extent of isotopic enrichment was analyzed by mass spectrometry by identification of $Cl(C_6H_4)CHCHCO$ ion fragments. The observed m/e ratios of the unlabeled fragments were 165.0109 and 167.0080 for ^{35}Cl and ^{37}Cl containing species (the corresponding calculated ratios are 165.0107 and 167.0077). Comparison of unlabeled and

^{17}O-enriched product by mass spectrometry showed ^{17}O enriched to 14.2% at the carbonyl oxygen position of the ester bond (theoretical limit of enrichment 21.5%). For the quantities employed, this represents ∿66% efficiency of incorporation of ^{17}O.

N-(benzoyl)-(glycyl-1-^{17}O)-L-phenylalanine. The procedure is based on the synthesis described by Antonovics and Young (5). To a solution of N-benzoyl-glycine (0.5 g, 2.79 mmole) in 10 ml THF, ^{17}O-enriched water (0.2 ml, 52.79% ^{17}O, 41.79% ^{18}O) was added with 0.005 ml of conc HCl (Baker Ultrex). The mixture was refluxed for 2 days. By slow evaporation of the solvent and drying over P_2O_5, all of the N-benzoyl-glycine was recovered as a white crystalline product.

To a solution of the $H_2{}^{17}O$ treated benzoyl-glycine dissolved in 10 ml of THF, a 10 ml solution (THF) of the hydrochloride salt of L-phenylalanine methylester (0.575 g, 2.79 mmole) containing TEA (0.38 ml, 2.79 mmole) was added slowly at ambient temperature. The mixture was cooled to -10°C, and dicyclocarbohexyldiimide (0.575 g, 2.79 mmole) suspended in 1 ml of THF was slowly added dropwise. The reaction mixture was allowed to warm to ambient temperature and was then stirred for 18 hours. A few drops of glacial acetic acid were then added, followed by stirring for 30 minutes. The urea precipitate was removed by filtration and the product recovered by solvent evaporation in vacuo. This residue was dissolved in chloroform; the organic layer was washed with 1 N HCl, saturated $NaHCO_3$, and saturated NaCl; and dried over anhydrous Na_2SO_4. Removal of the chloroform solvent in vacuo yielded a clear oil.

To the oil suspended in 6 ml of methanol was added 2 ml 1 N NaOH, followed by stirring for 2½ hours. The mixture was acidified with conc HCl. Upon evaporation of the methanol, the product was extracted into ethylacetate, followed by extraction into saturated $KHCO_3$. The aqueous suspension was acidified, the product filtered, extracted into ethylacetate, washed with 1 N HCl and saturated NaCl, followed by drying over Na_2SO_4. Evaporation of the ethylacetate yielded a clear oil. The oil was seeded with a few microscopic crystals of unlabeled N-(benzoyl)-glycyl-L-phenylalanine. Large white crystals of the product formed overnight. The crystals were recovered by filtration and dried in vacuo (0.454 g, 1.39 mmole, 50% yield; m.p. 136-139°C). Mass spectrometric

analysis of the parent molecular ion with an m/e ratio of 326 for the unlabeled compound showed ^{17}O isotopic enrichment to ~29%. The calculated maximum possible extent of enrichment was 35.2 g-atom %.

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REFERENCES

1. Sugawa, T., Kawada, Y., Katoh, M., and Iwamura, H., Bull. Chem. Soc. Japan **52:** 3391 (1979).
2. Valentine, B., St. Amour, T., Walter, R., and Fiat, D., Org. Magn. Resonance **13:** 232 (1980).
3. Kuo, L. C. and Makinen, M. W., J. Biol. Chem. **257:** 24 (1982).
4. Suh, J. and Kaiser, E. T., J. Am. Chem. Soc. **98:** 1940 (1976).
5. Antonovics, I. and Young, G. T., J. Chem. Soc. **1967C:** 595.