PREPARATION OF CHLOROACETALDEHYDE-1-^{14}C AND ITS REACTION WITH ADENINE AND CYTOSINE NUCLEOTIDES

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SUMMARY

Chloroacetaldehyde, which reacts selectively with cytosine and adenine nucleotides to give fluorescent etheno-bridged derivatives, was synthesized bearing a ^{14}C label. The chloroacetaldehyde 1-^{14}C, prepared from glycerol-2-^{14}C, was used to form etheno-bridged adenine and cytosine nucleotides for enzyme studies and was applied to the chemical modification of tRNA.

Key Words: 3-Chloro-1,2-propanediol, Chloroacetaldehyde, Carbon-14, Adenosine Phosphates, Cytidine Phosphates, Yeast $tRNA^{Phe}$

INTRODUCTION

The reaction of chloroacetaldehyde with adenine and cytosine derivatives to form etheno-bridged products [1,2] has found particular application at the nucleotide level. The fluorescent etheno-bridged adenine and cytosine nucleotides have been utilized as substitute cofactors with various enzymes, and the chloroacetaldehyde reaction has been used to modify nucleic acids [3]. In the latter case, the fluorescence of a 1,$\underline{N}^6$-ethenoadenylate unit is severely quenched in

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a polynucleotide. The synthesis of chloroacetaldehyde containing a radioactive label would allow more sensitive assay of cofactor-enzyme binding and more sensitive detection of the chemical modification of nucleic acids when the observed fluorescence is too low for quantitation. Accordingly, a synthetic scheme was devised to furnish chloroacetaldehyde-1-^{14}C (I).

EXPERIMENTAL

Chloroacetaldehyde-1-^{14}C (I). A solution of 1.0 mCi of glycerol-2-^{14}C (II) in 2-propanol/water, as received from Mallinckrodt, was stored at -20° until used. The labelled material was transferred by syringe, with three 1-ml methanol rinses of the vial and syringe, into a tared 10-ml centrifuge tube. The solution was evaporated under a stream of nitrogen while the tube was warmed in hot water, leaving 7.18 mg of residue. To this, 87 mg of redistilled glycerol was added to give a total of ca. 1 mmol. After addition of 0.315 ml of conc HCl and 0.12 ml of glacial acetic acid to the glycerol, the resulting clear solution was heated 10 hr under reflux on a steam bath using a cold-finger condenser. Next, the solution was evaporated under a stream of nitrogen while being warmed in a beaker of hot water. The residue was dissolved in 1.5 ml of methanol. Gas chromatography using a 3 ft x 1/4 in Porapak Q column at 230° (injection port, 305°; detector, 330°; He flow rate, 150 ml/min) showed one major component (III), with minor amounts of glycerol and dichloro compounds. It was decided to forego further purification by gas chromatography at this stage.

The solution containing mainly 3-chloro-1,2-propanediol-2-^{14}C (III) was transferred to a ground-glass stoppered tube, reduced to dryness as described above, and 2.0 ml of water was added. While the solution was stirred magnetically at 25°, 350 mg (1.5 mmol) of $NaIO_4$ was added in small portions over the course of 1 hr. After stirring an additional hour, the solution was distilled using a high

vacuum line. The resulting aqueous chloroacetaldehyde was purified by preparative gas chromatography using a Porapak Q column at 170° (injection port, 160°; detector, 200°; He flow rate, 120 ml/min), and the effluent was collected in a tared trap cooled in liquid nitrogen. The yield of chloroacetaldehyde-1-^{14}C, as the hydrate, was 42 mg (47% from glycerol). The material in the trap was transferred on a high vacuum line to a break-seal vessel for each of the reactions described below.

Reaction of Adenine and Cytosine 5'-Ribonucleotides with Chloroacetaldehyde-1-^{14}C. A mixture of 8 mg each of adenosine mono-, di-, and triphosphates (AMP, ADP, and ATP) in 0.1 M sodium citrate buffer (pH 5.0) was treated with the labelled $ClCH_2CHO$ for 48 hr at 25°. The resulting mixture of nucleotides and modified nucleotides was separated by ion exchange chromatography with DEAE Sephadex A-25 using a 0.5 → 2 M ammonium formate linear gradient (pH 4.2). Complete separation of all six components was achieved. The salt was removed under high vacuum from the pooled fractions, and the specific activity was measured. A solution of labelled 1,N^6-ethenoadenosine 5'-phosphate (εAMP-8-^{14}C) having a concentration of 7.20 x 10^{-2} mmol/ml as determined by UV [4] was counted by liquid scintillation in Aquasol (New England Nuclear). Chemical quenching was corrected by the Bush channels-ratio method [5]. Counting efficiency was approximately 80%. The specific activity of the εAMP-8-^{14}C was 0.62 μCi/μmol.

A mixture of cytidine mono-, di-, and trisphosphates (CMP, CDP, and CTP) was treated similarly with the labelled $ClCH_2CHO$ for 48 hr at 23°. The mixture was partially separated into the mono-, di-, and triphosphates of cytidine and labelled 3,N^4-ethenocytidine by the gradient chromatographic procedure described above.

Reaction of Yeast $tRNA^{Phe}$ with Chloroacetaldehyde-1-^{14}C. In a pilot experiment, separate solutions of yeast $tRNA^{Phe}$ (Boehringer-Mannheim), 1 mg in 0.01 M sodium cacodylate buffer at pH 6.0, were

successively exposed to the labelled reagent for periods of 1, 4, and 16 hr at 25° with stirring. The transfer of labelled chloroacetaldehyde was performed on a vacuum line. Partial enzymatic digestion and chromatographic separation of the fragments that reacted with chloroacetaldehyde, as outlined previously [3], can be followed by radioactivity.

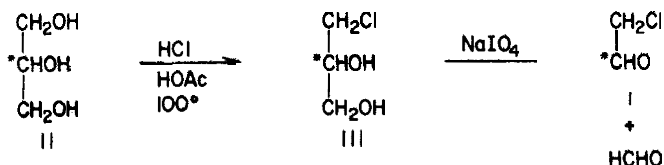

Figure 1

RESULTS AND DISCUSSION

The synthetic route to chloroacetaldehyde-1-^{14}C (I) is shown in Figure 1. Glycerol-2-^{14}C (II), when treated with concentrated hydrochloric acid and acetic acid according to the method of Rider and Hill [6] produced the intermediate 3-chloro-1,2-propanediol-2-^{14}C (III) in 80% yield. 3-Chloro-1,2-propanediol generally labelled with ^{14}C [7] might also be selected to serve as a precursor for ^{14}C-labelled chloroacetaldehyde. The intermediate III, upon examination by gas chromatography on Porapak Q, showed only trace amounts of the starting material and dichloro compounds. The diol III was cleaved with sodium metaperiodate [8] to produce the desired chloroacetaldehyde-1-^{14}C (I) in 47% overall yield after gas chromatographic purification to remove formaldehyde contaminant. The labelled chloroacetaldehyde was dissolved in water, and a 20% solution was used for the modification of ribonucleotides and tRNA.

The specific activity of the chloroacetaldehyde I was assayed by partial conversion to labelled 1,$\underline{N}^6$-ethenoadenosine 5'-phosphate

(labelled εAMP). The purified product was found to have a specific activity of 0.62 μCi/μmol, less than the activity of the starting material (1.0 μCi/μmol based on the supplier's specifications). The lower specific activity of the product could be the result of a lower total activity of the starting material II as supplied, and/or the starting material was radiochemically impure, containing some ^{14}C in the methylene carbons that would have been lost in the formation of compound I.

In order to make ^{14}C-labelled 1,$\underline{N}^6$-ethenoadenosine mono-, di-, and triphosphates, it was first ascertained that the unlabelled compounds could be separated chromatographically on DEAE Sephadex A-25 using a 0.5 → 2 M ammonium formate linear gradient at pH 4.2. Thus, it was possible to minimize the handling of the volatile chloroacetaldehyde-1-^{14}C by converting an intentional mixture of AMP, ADP, and ATP to a mixture of labelled εAMP, εADP, and εATP and then separating all six components in one chromatographic operation. In the event that only one of the adenosine phosphates had been selected for modification with chloroacetaldehyde, a chromatographic purification would still have been required, so the chromatographic procedure that we have described for the separation of starting material and product mixtures provides great simplification in the adenosine phosphate case. The separation in the case of chloroacetaldehyde modification of a mixture of CMP, CDP, and CTP was partially successful using gradient chromatography on DEAE Sephadex, and here intentional mixing of substrates may not be indicated.

The fluorescent modification of tRNA with chloroacetaldehyde and the location of the specific sites in the tertiary tRNA structure where chloroacetaldehyde attacks an adenosine or cytidine moiety offer many possibilities for biochemical and structural study [3]. The use of chloroacetaldehyde containing a radioactive label in reaction with specific tRNA's, illustrated here in a simple pilot experiment with yeast $tRNA^{Phe}$, allows more sensitive

detection of the chemical modification of nucleic acids. Finally, chloroacetaldehyde reverts the *Salmonella* bacterial tester strain TA 100 of Ames [9-11] and thus is a frameshift mutant. The labelled chloroacetaldehyde may be useful in attempting to relate mutagenicity and molecular targets in living systems.

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