SYNTHESIS OF OSSEOUS SPECIFIC ^{32}P-LABELED DISODIUM ETHANE-1-HYDROXY-1,1-DIPHOSPHONATE

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SUMMARY

High specific activity ^{32}P-labeled disodium ethane-1-hydroxy-1,1-diphosphonate has been prepared for specific use as a radiotherapeutic ameliorative treatment of osseous tumors by reacting ^{32}P-labeled phosphorus trichloride, acetic acid and water. This compound has been purified by crystallization of the disodium salt from a water-ethanol solution. Product identity and purity were established by reverse isotope dilution analysis. Spectrochemical quantitation, thin layer chromatography, radionuclide beta energy and half-life determinations are presented as essential procedures for establishing chemical and radiochemical purity when human distribution studies are planned.

Key Words: Phosphorus-32, phosphorus-33, synthesis, diphosphonate, bone tumors.

INTRODUCTION

Past therapeutic efforts have utilized orthophosphate-^{32}P or condensates of orthophosphate such as pyrophosphate-^{32}P or polyphosphate-^{32}P for amelioration of bone pain caused by primary and secondary bone tumors (1,2,3,4). However, the inherent in vivo limitations associated with the phosphates, such as susceptibility to enzymatic hydrolysis (5,6), and exchange of phosphate in the extracellular and cellular fluid and bone (7), has resulted in lower than desirable specificity to the tumor site. These problems necessitate repeated doses over a protracted period of time and hence increase radiation exposure and greatly lengthen patient treatment time.

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In recent years, research has been focused upon the specific affinity of disodium ethane-1-hydroxy-1,1-diphosphonate (EHDP) for calcium hydroxyapatite, the main constituent of bones and teeth (8-12). It is the ability of EHDP to chemisorb to hydroxyapatite (8) and particularly to sites of proliferating bone that forms the basis for the diphosphonate bone scanning agents (13-16). The use of EHDP to maintain or reinstitute calcium homeostasis in several disease processes associated with pathologic calcification has also been shown in both animals and man (17-22).

In light of these studies it seems appropriate to consider phosphorus-32 or phosphorus-33 radiolabeled EHDP with their respective monoenergetic 1.7 MeV beta and 0.25 MeV beta emission as possible therapeutic agents for the inhibition or destruction of bone tumors, both primary and metastatic. We feel that the choice of EHDP-^{32}P or EHDP-^{33}P for application to bone tumor treatment will depend upon intertrabecular distances in the specific bone tumor versus normal bone and upon sites of hemopoiesis and the relation of these to the effective range of the beta emission.

Distribution studies of EHDP-^{32}P in animals (7,23) and man (24,25) and EHDP-^{33}P in animals (7) have been recently reported.

Several methods of synthesis of EHDP have been reported (26-29) as has the use of EHDP for antitumor activity as EHDP-^{32}P and EHDP-^{33}P (30). The purpose of this paper is to present a method of synthesizing high specific activity phosphorus labelled EHDP compounds for human and animal therapeutic use.

MATERIALS AND METHODS

I. Synthesis

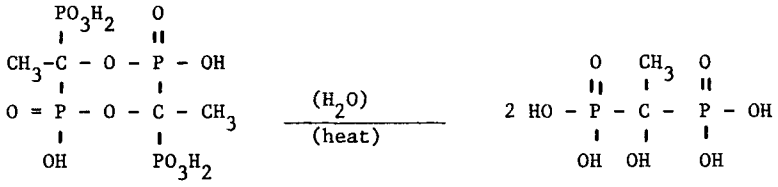
The more readily available phosphorus-32 isotope was initially utilized to synthesize labelled diphosphonate. Phosphorus-32 trichloride (4 mCi/mM) was purchased from Amersham-Searle Corporation, Arlington Heights, Illinois. Untagged PCl_3 and acetic acid were reagent grade chemicals obtained from Matheson, Coleman & Bell, and from Matheson Scientific, respectively. All glassware and equipment were standard laboratory equipment with the exception of a Dewar Dry Ice condenser (Ace Glass Co., Vineland, New Jersey, Cat. No. 9197-08) which was altered by shortening the outer jacket to reduce the clearance between the cold finger tip and the outer jacket to 0.5-1.0 mm for more efficient condensation of the small quantities of vapor. Also the 14/22 ℨ inner joint on this condenser was replaced with a 14/35 ℨ joint with a drip tip to return the condensate directly to the reaction mixture in the center of the pot. In the case of phosphorus-33 labelled phosphonate, the $^{33}PCl_3$ (2-8 mCi/mM) was synthesized from $H_3{}^{33}PO_4$ by an independent laboratory (International Chemical and Nuclear Corporation, Irvine, California). Subsequent batches of $^{33}PCl_3$ were obtained from Oak Ridge National Laboratories (31). The complete procedure for synthesis of the radioactive phosphorus labelled diphosphonate consisted of three steps: reaction, hydrolysis, and isolation.

(1) Reaction. A dry 25 ml three neck (14/20 ℨ) round bottom flask was fitted in the center neck with a Truebore stirrer with a Teflon half-moon blade (magnetic bar stirrers are generally inadequate

in this application). One side neck was fitted with a modified Dewar cold finger dry ice condenser which was filled with dry ice and ethanol. The remaining neck was closed with a glass stopper, except during the water addition step, when a rubber septum was used. The flask was immersed (only 1-2 cm) in a room temperature silicone oil bath. All joints were lightly lubricated. $^{32}PCl_3$ (0.80 ml, 9.4 mM, 37 mCi) was quickly poured into the open neck of the flask via a burette funnel and rinsed in with 0.60 ml (0.01 moles) of glacial acetic acid. The funnel was immediately replaced with a rubber serum stopper, and gentle agitation was started (100 RPM). Water (0.3 ml) was then added dropwise with a glass micropipette through the rubber septum over a five minute period. The bath temperature was increased gradually, reaching 60-70°C at 30 min, 120°C at 1 hr, and 145-148°C at 2 hrs, and was held constant until completion of the reaction at about 6 hours. The mixture became viscious at 1-2 hrs. Agitation was continued until the product solidified. A creamy gel appeared at 3-4 hrs, and a dry, white solid formed at 5.5 hrs. During the latter stages of the reaction, the cold tip of the dry ice condenser became covered with white crystals, presumably excess acetic acid.

(2) Hydrolysis. After 6 hours the dry ice condenser was replaced with an Allihn condenser cooled with tap water, and 8 ml of distilled water was carefully added to the reaction flask. The immersion depth was increased to about 1/3-1/2 of the flask diameter in the still hot (145-148°C) bath. One Hengar granule was added and the solution was

then refluxed rather vigorously for 40 hours. The initial product consisted of a mixture of EHDP cyclic condensates, principally the condensate shown below (I) which upon refluxing in aqueous solution was converted to the desired monomer (II).



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(3) Isolation. After hydrolysis was completed, the system was opened and 80-90% of the liquid was evaporated; 10 ml more water was added and the evaporation step was repeated. The solution was transferred to a volumetric flask and diluted to 25 ml with water. A 0.10 ml aliquot was further diluted to 50.0 ml for ^{32}P assay and for reverse isotope dilution (RID) analysis. The RID analysis (see Section II) prior to isolation of the product indicated the quantity of EHDP and thus helped to determine the amounts of water and ethanol needed for good crystallization yields. The pH of the remaining 24.9 ml of reaction solution was adjusted from approximately 1 to 5.0 by the addition of sodium hydroxide (8.3 mM required), and distilled water was added to make 49 ml (12.9 ml of H_2O/mM EHDP), based on the RID analysis of the product. To this solution, 121 ml of ethanol (formula 3A, anhydrous)

was added slowly with vigorous agitation to precipitate EHDP as the disodium salt of the acid (II). Stirring was continued for 30 min following ethanol addition. The solution was then vacuum filtered through a coarse porosity glass frit and washed with ethanol and ethyl ether. The crystals of EHDP·$4H_2O$-^{32}P were spread and air dried overnight at room temperature. The purity can be further improved by redissolving in H_2O and reprecipitating with alcohol.

II. Product Analysis

The radiochemical purity of the final product was determined by RID analysis. A series of dissolutions and recrystallizations of the product were carried out in order to obtain radiochemical purity greater than 95% as indicated by the results of the RID analysis. The specific activity of the EHDP-^{32}P or EHDP-^{33}P was determined by beta counting of aqueous solution aliquots in 10 ml of Cab-O-Sil gel using a Packard Liquid Scintillation Spectrometer. Additional methods have been used on subsequent batches of EHDP-^{32}P and EHDP-^{33}P to test the chemical, radiochemical and radionuclidic purity of the synthesis products where quality assurance of the final product was required for animal and human distribution studies (7, 23-25). These methods included thin layer chromatography (TLC), spectrophotometric titration, radiation half-life determination and radiation energy measurement.

In the TLC procedure, different concentrations of the product were spotted and separated on cellulose TLC sheets [solvent: p-toluenesulfonic acid monohydrate (3g), water (60 ml), acetone (25 ml),

tetrahydrofuran (60 ml), isobutanol (80 ml)] so that both major and minor components were visualized by the molybdenum blue reaction (32,33). Quantitative separations of EHDP, pyrophosphates and orthophosphate can be obtained by these methods. Comparison of the Rf, size and color intensity of the visualized spots in the sample to those of pure EHDP standards confirmed product identity and chemical purity. Scanning with an end window detector, or liquid scintillation counting of segmented strips, was done to show radiochemical purity.

The chemical concentration of EHDP was determined spectrophotometrically in dilute solutions (34). A thorium-cyclohexanediaminetetraacetic acid (Th-CyDTA) binary complex was used in the titrant for EHDP using xylenol orange as indicator. A ternary species, $TH_2(CyDTA)_2$ EHDP is quantitatively formed during titration before a weaker xylenol orange indicator complex $Th_2(CyDTA)_2(xylenol\ orange)_2$ is formed and a color change occurs. The endpoint was compared spectrophotometrically to pure EHDP standards.

The radionuclidic identity and purity of the phosphorus-32 was verified by the half-life and beta energy values. Radioactive decay of the EHDP-^{32}P was determined over two or more half-life periods; agreement with the known half-life (14.3 days) of ^{32}P confirmed identity and purity of the radionuclide. Measurement of the characteristic monoenergetic 1.71 MeV beta particle energy through the use of an end window Geiger counter and low molecular weight (aluminum) absorbers defined the nuclide. The experimentally determined absorber half-thickness for EHDP-^{32}P was compared to that of a pure ^{32}P standard;

equivalent results indicated high radionuclidic purity for the product (the half-thickness value obtained for pure ^{32}P under the prevailing test conditions was 102 ± 5 mg/cm^2). Similar identity and purity methods were used when the nuclide was phosphorus-33.

RESULTS

The synthesis produced 2.87 mM of $Na_2EHDP \cdot 4H_2O$-^{32}P (approximately 65% of theory) having a specific activity of 8.4 mCi/mM of EHDP. Reverse isotope dilution analysis indicated greater than 95% purity without a second purification precipitation step.

DISCUSSION

The method described in this paper is a modification and scale down of our initial procedure to produce high purity EHDP-^{32}P for chemical and metabolic studies (8,10). The current procedure produced high specific activity EHDP-^{32}P or EHDP-^{33}P for possible therapeutic and ameliorative applications. Much higher specific activity EHDP-^{32}P (up to 190 mCi/mM) has been subsequently achieved by contracting with an independent laboratory* and using the above method of synthesis. In these subsequent batches used for animal and clinical applications (7, 23-25) all the above product analysis procedures have been employed.

The specific affinity of EHDP for bone and its in vivo stability should permit the use of highly purified, high specific

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activity ^{32}P- or ^{33}P-labelled compound as a therapeutic or palliative treatment for malignant osseous disorders in humans, especially if detected at an early stage of osseous involvement.

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