THE PREPARATION OF $H_2{}^{18}O_2$ IN AQUEOUS SOLUTION

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Summary: A method is described for the preparation of [^{18}O]-hydrogen peroxide in an aqueous solvent of natural isotopic abundance through the autoxidation of 2-ethyl anthrahydroquinol with gaseous $^{18}O_2$.

Keywords: hydrogen peroxide, ^{18}O-labelling, ^{2}H-labelling

1. Introduction:

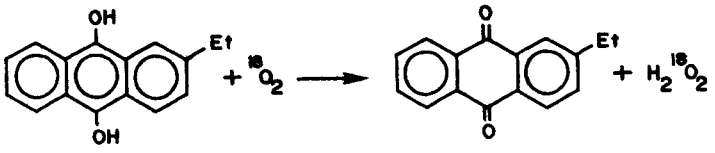
Isotopic labeling of hydrogen peroxide is important for the investigation of peroxide reaction mechanisms. Isotopically labeled hydrogen peroxide in aqueous solution is frequently used in the study of the catalytic peroxide decomposition mechanisms of various enzymes (1-5). A number of syntheses exist which can be used for the preparation of isotopically labeled H_2O_2 (6-10). Unfortunately, many of these methods also allow for the production or presence of labeled water. Most of the methods which remain are either somewhat difficult to perform or have relatively low yields.

Production of hydrogen peroxide by the autoxidation of 2-ethyl anthrahydroquinol is a common industrial procedure (11). We have modified this reaction for the production of small quantities of aqueous $H_2{}^{18}O_2$ needed for enzymatic studies, minimizing contamination from organic substances. The experimental procedure for this reaction

0362-4803/85/050461-05$01.00

Received September 25, 1984

described here is easy to perform with ordinary laboratory equipment and readily available gaseous $^{18}O_2$. In addition to the preparation of $H_2{}^{18}O_2$, the procedure also affords the preparation of $^2H_2{}^{16}O_2$ and $^2H_2{}^{18}O_2$ with the substitution of molecular deuterium for the molecular hydrogen. Using 99% pure $^{18}O_2$, mass spectral analysis showed the hydrogen peroxide produced to be over 95% doubly labelled.



2. Materials

The 2-ethylanthraquinone (Aldrich Chemical Co., Milwaukee, Wisc.) was recrystallized twice from boiling acetic acid, washed with cold ethanol and dried in a vacuum over phosphorous pentoxide (Fisher Scientific Co., Fairlawn, NJ). The solvent, 1-decanol (Aldrich), was vacuum distilled three times through a 22 cm column packed with glass beads. The 5% palladium on alumina catalyst was also obtained from Aldrich. 99% purity $^{18}O_2$, in a 250 ml glass bottle with a side arm and breakable glass seal, was obtained from Stohler Isotope Chemicals (Waltham, Mass.). The palladium and $^{18}O_2$ were both used as received.

3. Experimental

A 6% solution (w/w) of 2-ethylanthraquinone (4.2 g, 1.77×10^{-2} M) in 1-decanol (84 ml), with a catalytic amount of 5% palladium on alumina, was hydrogenated overnight (approximately 12 hours) in a catalytic hydrogenation apparatus (Parr Corp., Moline, Ill.) at room temperature under a hydrogen pressure of 2 atm. The reaction bottle was then placed in a nitrogen purged glove bag.

The following operations were performed within the glove bag. The reaction bottle was opened and the reaction mixture filtered through a fritted glass funnel (medium porosity) to remove the palladium. The resulting solution of 2-ethyl anthrahydroquinol (fluorescent yellow-green) was quickly poured into the autoxidation vessel. The autoxidation vessel consisted of an Erlenmeyer flask with the $^{18}O_2$ bottle attached by a glass seal via the bottle side arm. The mouth of the Erlenmeyer was closed with a rubber septum and the container was flushed with N_2 using two hypodermic needles inserted in the rubber septum. One hypodermic needle was the N_2 inlet. The other hypodermic needle was the outlet and was unattached. The vessel was kept slightly pressurized by removing the outlet needle a few seconds before the inlet needle. The seal on the $^{18}O_2$ container was then broken using a glass encased metal bar (magnetized) placed previously in the autoxidation vessel. The autoxidation was accomplished by stirring and took approximately 60 minutes at room temperature proceeding through a brown intermediate.

Once the reaction mixture returned to the bright yellow color of 2-ethylanthraquinone in solution, the mixture was removed from the glove bag and transferred to a separatory funnel. The hydrogen peroxide was extracted from the decanol with four equal volumes of chilled (4°C) high purity deionized water (5 ml). Approximately 8% of the water used in the extraction was retained by the reaction mixture which agrees with earlier observations (12).

Unreacted 2-ethyl anthrahydroquinol could result in the formation of hydrogen peroxide of natural isotopic abundance when the reaction vessel is opened to the external atmosphere. To ensure that no unreacted 2-ethyl anthrahydroquinol remained after the autoxidation procedure, the quantity of the reaction mixture was adjusted assuming an 80% yield based on the available $^{18}O_2$ (1.02×10^{-2} moles).

Titration of the $H_2{}^{18}O_2$ produced with 0.01 N permanganate showed a 90% yield (7.3×10^{-3} moles in 18.3 ml) based on a theoretical yield of 1.26×10^{-1} moles $H_2{}^{18}O_2$/liter (8) and an overall yield of 72% based on the $^{18}O_2$ available. Mass spectral analysis (against a 0.30 M $H_2{}^{16}O_2$ standard) showed the atom percent of ^{16}O in H_2O_2 to be less than 5 percent. The mass spectral analysis also showed no contaminating organic compounds. In our laboratory the samples were diluted for use in enzymatic studies, however concentration can be performed by low pressure fractionation (13). The oxygens in hydrogen peroxide do not readily exchange with the oxygen atoms of water. Our samples stored at 4 °C maintained their isotopic purity at least for several weeks.

Acknowledgements:

Financial support is acknowledged from the Research Corporation, the donors of the Petroleum Research Fund as administered by the American Chemical Society, and the Jeffress Memorial Trust. We are grateful to Dr. Richard Kornfeld of the Philip Morris Research Center, Richmond, Virginia for the use of Mass Spectroscopy facilities and to Dr. Rickey P. Hicks for helpful suggestions.

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