RADIOSYNTHESIS OF ^{15}O-LABELED HYDROGEN PEROXIDE

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SUMMARY

^{15}O-H_2O_2 is thought to be a candidate of attractive injectable tracers for the study of oxygen metabolism with PET. A simple synthetic method yielding ^{15}O-H_2O_2 in saline solution by the autoxidation of 2-ethylanthrahydroquinol with gaseous ^{15}O-O_2 produced by cyclotron target system is described.

KEYWORDS: ^{15}O-H_2O_2, CMRO$_2$, PET, SEP-PAK^{TM} C$_{18}$

INTRODUCTION

In positron emission tomography (PET), oxygen metabolism is currently measured using ^{15}O-O_2 gas by continuous(1) or single inhalation(2). However, another chemical form of tracer which can be intravenously injectable is necessary in clinical PET field, because gaseous tracer sometimes makes it difficult to obtain steady respiration or to collaborate with patients with serious disease.

$^{15}O-H_2O_2$ is thought to be one of the candidates of such tracers. In the ideal decomposition of $^{15}O-H_2O_2$, ^{15}O radio-activities will be divided equally into $^{15}O-O_2$ and $^{15}O-H_2O$. In the PET study of $^{15}O-O_2$ inhalation, the $CMRO_2$ is calculated based on the kinetic model which includes administered $^{15}O-O_2$ and $^{15}O-H_2O$ produced in vivo(1,2). If we have a highly quantitative PET scanner, the mathematical model to calculate oxygen metabolism can be applicable after intravenous $^{15}O-H_2O_2$ administration.

A number of methods were reported which produced isotopically labeled H_2O_2 (3-8). However, the synthesis of ^{15}O labeled H_2O_2 has not been reported. It is well known that H_2O_2 is synthesized in high yield by the autoxidation of some reducing agents(7,8). We developed a method for the synthesis of $^{15}O-H_2O_2$ by the autoxidation of 2-ethylanthrahydroquinol with gaseous $^{15}O-O_2$ (Fig.1) in sufficiently short period as compared with the physical half life of ^{15}O (2 minutes).

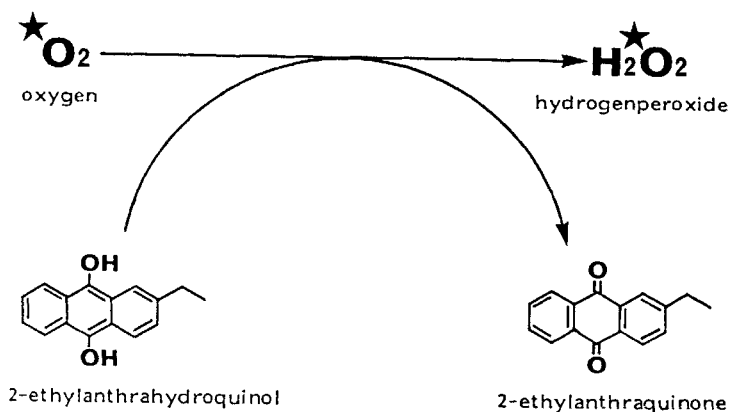Fig.1   Scheme for synthesis of $^{15}O-H_2O_2$

MATERIALS AND METHODS

preparation of 2-ethylanthrahydroquinol

2-Ethylanthraquinone (236mg,1.0mmol, Wako Pure Chemical Industries Ltd.) was dissolved in ethyl acetate (10mL) in a 30mL

vial. A magnetic stirring bar was placed in the vial and
palladium carbon (5%,5mg) was added to the solution. The vial
was closed with teflon laminated packing and an aluminum cap.
A bag filled with hydrogen gas (1L) was connected to the vial
with a needle (18G). Another needle was stuck into the vial and
the gas in the vial was exchanged with hydrogen gas by pushing
the bag until the resulting volume became 0.5 liter. The needle
which was opened to the room air was removed from the vial. Under
this condition, the mixture was stirred for 3 hours at room
temperature in order to reduce 2-ethylanthraquinone. The
resulting mixture contained 2-ethylanthrahydroquinol which was
confirmed by yellow-green fluorescence. The needle which led to
the hydrogen bag was removed from the vial.

analysis of 2-ethylanthrahydroquinol

The above solution containing 2-ethylanthrahydroquinol (1mL)
was placed in a test tube, and air (34mL,300μmol O_2) was bubbled
into the solution. The resulting solution was analized for H_2O_2
formed by autoxidation. The amount of 2-ethylanthrahydroquinol
was evaluated to be equal to the amount of H_2O_2, assuming that
H_2O_2 was produced from 2-ethylanthrahydroquinol quantitatively.

preparation of a reaction column

Two SEP-PAK C_{18} cartridges (Millipore Corporation) were
serially connected with a short polyethylene tube and both ends
were connected to disposable three way valves. The air in these
columns was swept out by passing helium gas through the columns
for 2 minutes. The solution containing 2-ethylanthrahydroquinol
(1mL) described above was carefully added to top of the column to
isolate the syringe and needle from air. The solvent, ethyl
acetate, was evaporated completely by purging with stream of
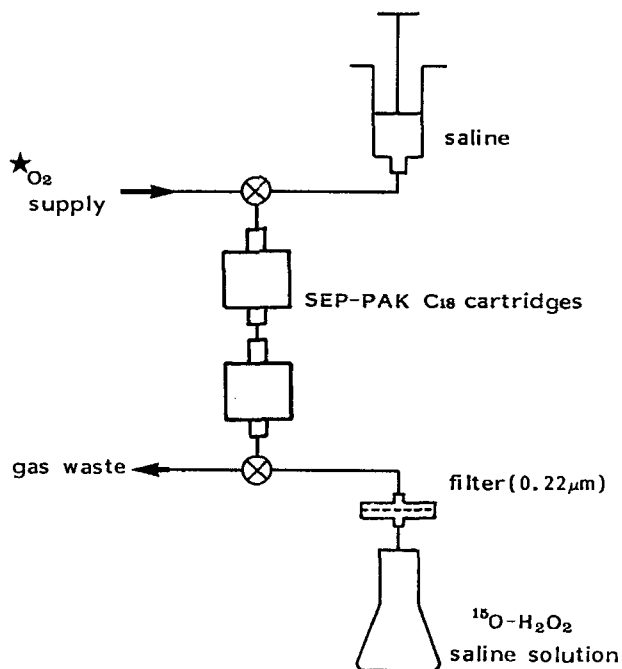helium gas through the columns for about 5 minutes.

Fig.2   Schema for ^{15}O-H_2O_2 synthetic procedure. SEP-PAK C_{18}
         cartridges contained 2-ethylanthrahydroquinol.

preparation of ^{15}O-H_2O_2 (Fig.2)

^{15}O-O_2 gas (ca 56GBq; 1.5Ci) was produced by the $^{14}N(d,n)^{15}O$
nuclear reaction in the target chamber which was filled with
nitrogen gas containing 0.5% oxygen gas. The target gas was
introduced to the columns containing 2-ethylanthrahydroquinol
described above at a flow rate of 500mL/min (500mL,100μmol).
After the radioactivity trapped in the columns had reached
saturation, saline (6mL) in a syringe was passed through the
columns and a sterile filter (0.22 μm, Millipore Corporation).

analysis of the products

The radiochemical purity was analyzed by HPLC and chemical
degradation by potassium permanganate (8). The HPLC was performed

with a Radial-PAK C_{18} column (Waters) eluted with a mixture of ethanol/water (20/80) at a flow rate of 2mL/min. The chemical degradation was carried out as follows. The ^{15}O-H_2O_2 solution (1mL) was mixed with permanganate (0.05N,1mL) and H_2SO_4 (1N,0.5mL). The mixture was heated at 60°C, after which air was bubbled into the mixture for 1 minute in order to remove ^{15}O-O_2 formed by the oxidation of ^{15}O-H_2O_2. The radiochemical purity was calculated as follows:

$$\frac{\text{the radioactivity loss as } ^{15}\text{O-O}_2}{\text{the initial } ^{15}\text{O-radioactivity}} \times 200$$

The amount of carrier H_2O_2 was determined by the comparison of the integrated UV absorption peak (λ=254nm) of the sample with that of authentic H_2O_2 on the HPLC.

RESULTS

The amount of 2-ethylanthrahydroquinol in the solution as mentioned above was determined. Twenty micromole of H_2O_2 was generated from 1.0 ml of the solution. Therefore the amount of 2-ethylanthrahydroquinol was 20μmol and the yield of 2-ethyl-anthrahydroquinol from 2-ethylanthraquinone (100μmol) was 20%.

The column containing 2-ethylanthrahydroquinol trapped ^{15}O radioactivity in 7% radiochemical yield. The trapped radioactivity was eluted with 6ml of saline in 75% yield based on ^{15}O radioactivity trapped in the columns. By the method reported here, we obtained ^{15}O-H_2O_2 in sterile saline solution within only 3 minutes after the end of bombardment in about 6% radiochemical yield based on ^{15}O-O_2 recovered from the target chamber(Table 1).

Table 1. Radiochemical Yield and Carrier of Each Stage

Resulting material	Yield[*](%)	Carrier(μmol)
2-ethylanthrahydroquinol		20
^{15}O-O_2	100	100
trapped ^{15}O-O_2 on the columns	7.3	(20)[Φ]
eluate from the columns	5.5	15

[*]Based on ^{15}O-O_2 recovered from the target chamber.

[Φ]Estimated value for oxygen reduced by 2-ethylanthra-hydroquinol. See discussion.

The radiochemical and chemical purities of ^{15}O-H_2O_2 in saline were analized. On HPLC, H_2O_2 and H_2O had the same retention time and the radioactive single peak was obtained which corresponds to them (Fig.3). There was no peak corresponding to other radioactive compounds such as ethyl acetate or acetic acid. The chemical degradation study showed that the radiochemical purity of ^{15}O-H_2O_2 was around 80%. Therefore, about 20% of ^{15}O radioactivity in the saline represented contamination by ^{15}O-H_2O. Carrier H_2O_2 was 0.5mg (15μmol) in each synthesis, and other chemical impurities with UV absorption (anthraquinone derivatives or ethyl acetate) were not detected by HPLC analysis (Fig.3).

DISCUSSION

In order to shorten the synthesis time and to increase the radiochemical yield, we used the micro column (SEP-PAK C_{18} cartridge) which was a modification of the captive solvent method (9) for the autoxidation reaction. Preceding the radiosynthesis, the column which contained 2-ethylanthrahydroquinol but no solvent was prepared. Thus, available radioactivies (>740MBq; >20mCi) for PET study can be obtained as ^{15}O-H_2O_2 within only 3 minutes after the end of bombardment.

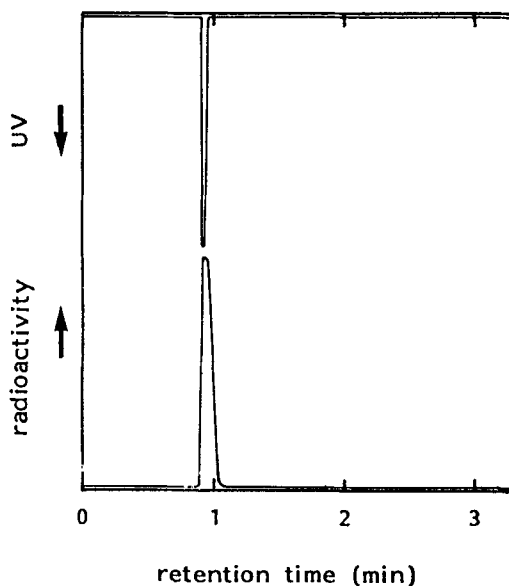Fig.3   HPLC analysis of produced ^{15}O-H${}_2$O${}_2$ solution.

The HPLC was performed with a Radial-PAK C${}_{18}$

(Waters) column eluted with a mixture of

ethanol/water(20/80) at a flow rate of 2mL/min.

2-Ethylanthrahydroquinol used in this synthesis was 20μmol
which was less than the amount of the carrier oxygen (100μmol).
The O${}_2$ reduced in the column was thought to be around 20μmol
because the carrier of produced H${}_2$O${}_2$ was 15μmol and the
radiochemical yield of the product was 75% based on the trapped
^{15}O-O${}_2$. Therefore, the yield of the product might be almost 100%
based on 2-ethylanthrahydroquinol. On the other hand, only 7% of
^{15}O-O${}_2$ was trapped in the column, though its percentage should be
20% (20μmol reduced/100μmol supplied). The discrepancy of ^{15}O-O${}_2$
trapping yield (Δ13%) was thought to be caused by the fact that
2-ethylanthrahydroquinol was already oxidized by air in the
atmosphere during the preparation of the column . In our previous
^{15}O-butanol synthesis using tributylborane (10), the air had no

effect on the yield, although the same procedure as that in this study was used for column preparation. One of the reasons for this difference   is considered that the amount of the hydroquinol (20μmol) used in this study is much smaller than that of the alkylborane (1.0mmol). The radiochemical yield of this 2-ethylanthrahydroquinol and prepare the reaction column under completely anaerobic condition.

The sterile saline solution of ^{15}O-H_2O_2 was synthesized by this method. However, in order to use this tracer in clinical PET, further studies to increase the radiochemical yield and the radiochemical purity are necessary as well as fundamental tracer kinetic studies in vivo.

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