[^{11}C]LABELING OF COENZYME Q_{10} AND ITS TISSUE DISTRIBUTION

T. Takahashi, T. Ido, R. Iwata, K. Ishiwata, K. Hamamura* and K. Kogure**

Cyclotron and Radioisotope Center, Tohoku University, Aramaki Aza Aoba, Sendai, 980, Japan
*Eisai Co., Ltd., Tsukuba Research Laboratories, 1-3 Tokodai 5-chome Toyosato-machi, Tsukuba-gun, Ibaragi, 300-26, Japan
**Department of Neurology, Institute of Brain Diseases, Tohoku University School of Medicine, 4-1 Seiryo-cho, Sendai, 980, Japan

SUMMARY

[^{11}C]Coenzyme Q_{10} was synthesized by O-methylation of 3-demethyl coenzyme Q_{10} with $^{11}CH_3I$. We have produced 1-4 mCi of [^{11}C]coenzyme Q_{10} with radiochemical yields of 6-16 % (based on trapping $^{11}CH_3I$) in 35-50 min with radiochemical purities of >95 %. The specific activity was 4-5 mCi/μmol at the end of [^{11}C]-coenzyme Q_{10} synthesis. The tissue distribution was investigated on mongolian gerbils after intravenous administration of [^{11}C]-coenzyme Q_{10} solubilized with polyoxyethylene hydrogenated caster oil (HCO-60). The blood level of [^{11}C]coenzyme Q_{10} was very high and its clearance was slow. The accumulations in the heart, kidney and liver were moderate and the accumulation in the brain was low in spite of its high lipophilicity.

Key words : [^{11}C]Coenzyme Q_{10}, O-Methylation, Tissue distribution

0362-4803/85/060565–11$01.10

Received July 30, 1984
Revised November 5, 1984

INTRODUCTION

Coenzyme Q_{10} (CoQ_{10}) is shown to function as one component of the electron transfer sequence in mitochondria (1,2) and to function as a lipophilic molecule shuttling between the flavoproteins and the cytochrome system in the lipid phase of the mitochondrial membrane (3). It has been proven that CoQ_{10} not only performs the electron transfer by moving about freely in the mitochondrial membrane but acts as an anti-oxidant toward the super-oxidative reaction in vivo (4). In these respects, CoQ_{10} is expected to be used as a therapeutic agent for various ischemia which are mainly caused by the deficiency of oxygen owing to the blocking of the electron transfer sequence. In practice, CoQ_{10} is used as a therapeutic agent for myocardial ischemia. By these characteristics, [^{11}C]CoQ_{10} may be applied to a PET study of myocardial ischemia. CoQ_{10} has been labeled with ^{3}H or ^{14}C in its side chain (^{3}H-Q_{10} or ^{14}C-Q_{10}) and their tissue distributions in rats and rabbits have been investigated (5,6). The main metabolites of ^{3}H-Q_{10} formed by ω-oxidation of its side chain, followed by β-oxidation, were 2,3-dimethoxy-5-methyl-6-(3'-carboxy-3'-methylpropyl)-1,4-benzoquinone and 2,3-dimethoxy-5-methyl-6-(5'-carboxy-3'-hydroxy-3'-pentylmethyl)-1,4-benzoquinone lactone (7). We tried [^{11}C]labeling the 3-methoxy group substituted in 1,4-benzoquinone. The [^{11}C]labeling of CoQ_{10} was carried out by O-methylation of 3-demethyl CoQ_{10} with $^{11}CH_3I$.

MATERIALS AND METHODS

Melting points are uncorrected. Infrared resonance (IR) spectra were determined on a Hitachi 260-30 spectrometer. Nuclear magnetic resonance (NMR) spectra and carbon-13 NMR spectra were determined on a JNM-FX-100 spectrometer. Both proton and carbon-13 chemical shifts are expressed in parts per million downfield

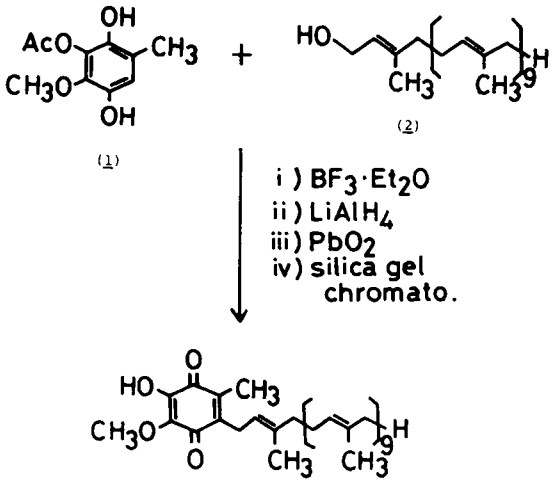


Fig. 1. Preparation of 3-demethyl CoQ_{10}

from the internal tetramethylsilane reference. Mass (MS) spectra were recorded on a JMS-01-SG spectrometer.

Preparation of 3-demethyl CoQ_{10} : 3-Demethyl CoQ_{10} was synthesized from 3-acetoxy-2-methoxy-5-methylhydroquinone 1 and decaprenol 2 according to the reaction shown in Fig. 1 (8,9). 3-Demethyl CoQ_{10} was given as a red oil. IR (neat) : 1615, 1640, 1660 (1,4-benzoquinone), 3380 (OH). MS m/e : 850 (M^++2), 221, 183. ^{13}C-NMR data of 3-demethyl CoQ_{10} is shown in Fig. 2.

O-Methylation of 3-demethyl CoQ_{10} : To a mixture of 85 mg (100 μmol) of 3-demethyl CoQ_{10} dissolved in 1 ml of N,N-dimethylformamide (DMF) and 47 mg (200 μmol) of Ag_2O, a solution of 43 mg (300 μmol) of CH_3I dissolved in 0.5 ml of DMF was added dropwise and the mixture was stirred at room temperature (20 °C) for 15 min. After Ag_2O/AgI was filtered off, the filtrate was poured into 5 ml of ice-water. The mixture was extracted with ether. The ether layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. 88 mg of CoQ_{10} was obtained. m.p. : 33-41 °C. IR (KBr) : 1610, 1650, 1660 (1,4-benzoquinone). ^{1}H-NMR ($CDCl_3$) : δ=1.60 (s; 27H; -(isoprenoid)-(CH_2-CH=trance=C($\underline{CH_3}$)-CH_2)$_9$-H), 1.65 (s; 3H;

-(isoprenoid)$_9$-CH$_2$-CH$\overset{cis}{=}$C(CH$_3$)-CH$_3$), 1.73 (s; 3H; -CH$_2$-CH=C(CH$_3$)-CH$_2$-(isoprenoid)$_9$-H), 1.98 (m; 39H; 5-CH$_3$ and -CH$_2$-CH=C(CH$_3$)-CH$_2$-(CH$_2$-CH=C(CH$_3$)-CH$_2$)$_8$-CH$_2$-CH=C(CH$_3$)-CH$_3$), 3.13 (d; 2H; -CH$_2$-CH=C(CH$_3$)-CH$_2$-(isoprenoid)$_9$-H), 3.92 (s; 6H; 2-OCH$_3$ and 3-OCH$_3$), 5.05 (m; 10H; -(CH$_2$-CH=C(CH$_3$)-CH$_2$)$_{10}$-H). ^{13}C-NMR data of CoQ$_{10}$ are shown in Fig. 2.

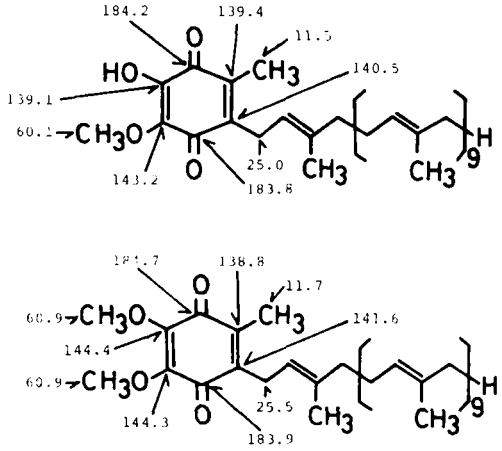


Fig. 2. ^{13}C-NMR of 3-demethyl CoQ$_{10}$ and CoQ$_{10}$ (solvent : CDCl$_3$, value : δ, unit : Hz)

Preparation of $^{11}CH_3I$: $^{11}CO_2$ was produced from the proton bombardment of nitrogen gas by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction. (cyclotron : CGR-MeV model 680, Tohoku University) $^{11}CH_3I$ was synthesized from $^{11}CO_2$ according to the following reaction scheme by the automated synthesis system (10) :

$$^{11}CO_2 \xrightarrow[-20\ °C]{LiAlH_4/THF} \xrightarrow[70\ °C]{THF\ \uparrow} \xrightarrow[-20\ °C]{H_2O} {}^{11}CH_3OH \xrightarrow[100\ °C]{HI} {}^{11}CH_3I$$

The total time required for the synthesis of $^{11}CH_3I$ from $^{11}CO_2$ was approximately 25 min.

Preparation of [^{11}C]CoQ$_{10}$: $^{11}CH_3I$ was trapped (flow rate : 200 ml/min, total time : 5-10 min) in 100 μl of acetone containing 8.5 mg (10 μmol) of 3-demethyl CoQ$_{10}$ and 4.7 mg (20 μmol) of Ag$_2$O

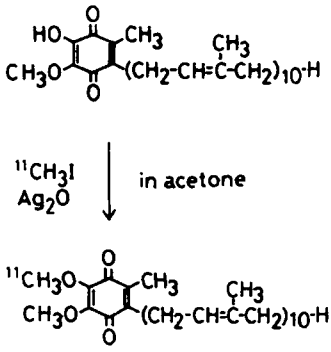
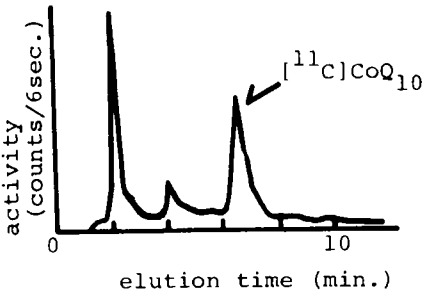


Fig. 3. Synthesis of [^{11}C]CoQ_{10}

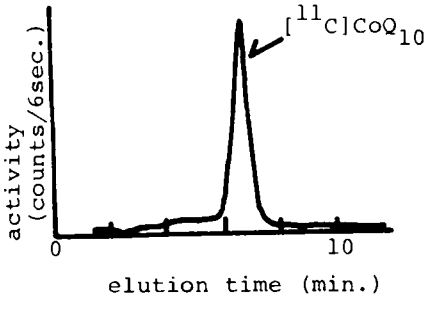
at -78 °C (dry ice-acetone). The reaction mixture was then warmed to 55-60 °C for 10 min with stirring. After removal of the solvent, the oily residue was dissolved in the solvent of n-hexane/ether=2/3 for chromatographic separation on a silica-gel column (silica-gel 60 (Merck), column : 1.1 cm i.d. x 10 cm, solvent : n-hexane/ether=2/3). [^{11}C]CoQ_{10} was eluted with 10-30 ml of the solvent. From the combined fractions, the solvent was removed (Fig. 3).

To a solution of the oily residue dissolved in 1 ml of benzene, 1 ml of 5 % polyoxyethylene hydrogenated caster oil (HCO-60) in benzene was added and the solvent was evaporated completely. To the residue, saline was added with shaking and the emulsion was filtered through a 0.22 μm pore size membrane filter for injection.

Radiochemical analysis of [^{11}C]CoQ_{10} : The radiochemical purity was determined by HPLC (column : μ-Bondapak C-18, eluent : MeOH/EtOH=3/2, flow rate : 2 ml/min). The peak of [^{11}C]CoQ_{10} was observed at the retention time of 6.3 min (Fig. 4).



Before passing through a silica-gel column



After passing through a silica-gel column

Fig. 4. Radiochemical analysis of produced [^{11}C]CoQ_{10} with HPLC. The radiochemical purity was analyzed before (upper) and after passing through a silica-gel column (lower).

Tissue distribution of [^{11}C]CoQ_{10} : [^{11}C]CoQ_{10} was injected into Mongolian Gerbils through a tail lateral vein. At 5, 10, 20 and 30 min post injection intervals, the animals were killed by neck dislocation. The organs and tissues were excised, rinsed, blotted to remove adhering blood and weighed. They were then counted in an automated NaI counter. The uptake is expressed as the differential absorption ratio (DAR). (DAR=[(the observed tissue activity) x (the body weight)] / [(the injected activity) x (the tissue weight)])

RESULTS AND DISCUSSION

O-Methylation with $^{11}CH_3I$ has been only applied to the synthesis of 3-[^{11}C]methyl-D-glucose (11). N-Methylation of many amino compounds easily proceeds with the addition of $^{11}CH_3I$ because of their basicity, but O-methylation does not proceed without addition of a base. In O-methylation of 3-demethyl CoQ_{10} with $^{11}CH_3I$, the addition of Ag_2O results in a good radiochemical yield of [^{11}C]CoQ_{10}. Using other bases (NaOH etc.) instead of Ag_2O, a chromene derivative was mainly obtained.

The effect of substrate concentration is important to the radiochemical yield in this reaction. Table 1 shows that a low concentration of 3-demethyl CoQ_{10} results in a low radiochemical yield of [^{11}C]CoQ_{10} and at concentrations above 100 μmol/ml, the radiochemical yield was enhanced. The use of excess 3-demethyl CoQ_{10} was disadvantageous in the separation of [^{11}C]CoQ_{10} and

Table 1. [^{11}C]Labeling conditions of CoQ_{10}

3-Demethyl CoQ_{10} mg (μmol)	1.0 (1.2)	1.3 (1.5)	5.7 (6.7)	8.5 (10.0)	8.5 (10.0)	8.5 (10.0)	8.5 (10.0)
Solvent (100 μl)	DMF	DMF	DMF	DMF	DMF	acetone	acetone
Concentration of 3-demethyl CoQ_{10} in solvent (μmol/ml)	12	15	67	100	100	100	100
[^{11}C]CH_3I (mCi)	80.4	90.4	89.0	72.6	80.9	150.2	83.4
[^{11}C]CoQ_{10} (mCi)*	0.3	0.9	3.9	4.3	5.5	23.8	11.5
Radiochemical yield (%)	0.4	1.0	4.4	5.9	6.8	15.8	13.8
Time required for the synthesis (min)	41	45	47	38	43	49	35

*) Radioactivity is corrected at the end of trapping [^{11}C]CH_3I.

unreacted substrate. So, the concentration of 100 μmol/ml results in the best radiochemical yield.

The trapping efficiency of $^{11}CH_3I$ was low in ice-water (0 °C) or at room temperature (20 °C). Acetone was the most suitable solvent because of the unfreezing at -78 °C and easy removal. The best reaction temperature was 55-60 °C which was slightly below the boiling point of acetone. The reaction time of 10 min was sufficient to obtain [^{11}C]CoQ_{10}.

HPLC analysis indicated that employing a silica-gel column was effective in removing the radioactive by-products (Fig. 4).

We have produced 1-4 mCi of [^{11}C]CoQ_{10} with radiochemical yields of 6-16 % (based on trapping $^{11}CH_3I$) in 35-50 min with radiochemical purities of >95 %. The specific activity was 4-5 mCi/μmol at the end of the [^{11}C]CoQ_{10} synthesis. This method may be widely applied to the [^{11}C]labeling of other phenolic compounds.

Table 2 indicated that [^{11}C]CoQ_{10} was cleared very slowly from the blood and was retained in blood at the highest level at the 30 min interval after injection. The increasing accumulations in the gall bladder and kidney show the excretion of [^{11}C]CoQ_{10} to feces and urine. The accumulation in the brain increased with time but was low in spite of the high lipophilic property of CoQ_{10}. Though the accumulation in the heart was moderate,it was retained for a prolonged period because of the large quantity of CoQ_{10} in the blood. The moderate heart uptake, the low brain uptake and the slow blood clearance of [^{11}C]CoQ_{10} are similar to those of ^{3}H-Q_{10} reported by Fujita T. et al (5). The myocardial imaging study by combining a [^{11}C]CoQ_{10} scan with a [^{45}Ti]DTPA scan ($T_{1/2}$=3.09 hr, indicator of blood volume) in a dual radionuclide subtraction technique is presently in progress.

Table 2. Tissue distribution of [^{11}C]CoQ_{10} and ^{3}H-Q_{10}

[^{11}C]CoQ_{10}

Tissue	Uptake*			
	5 min	10 min	20 min	30 min
Blood	15.96 ± 0.68	14.09 ± 0.71	13.30 ± 2.94	13.34 ± 1.49
Brain	0.32 ± 0.03	0.33 ± 0.01	0.34 ± 0.14	0.46 ± 0.12
Heart	1.82 ± 0.19	1.27 ± 0.03	1.70 ± 0.99	1.53 ± 0.22
Lung	4.49 ± 0.85	5.17 ± 0.02	3.51 ± 1.04	4.81 ± 1.28
Liver	1.22 ± 0.06	1.58 ± 0.14	0.98 ± 0.05	1.35 ± 0.20
Gall bladder	0.48 ± 0.27	1.79 ± 1.40	1.77 ± 1.13	2.15 ± 0.81
Spleen	1.98 ± 0.25	1.75 ± 0.23	1.31 ± 0.35	2.31 ± 1.18
Kidney	1.63 ± 0.14	1.82 ± 0.31	1.10 ± 0.17	1.71 ± 0.28
Muscle	0.15 ± 0.03	0.17 ± 0.07	0.26 ± 0.14	0.24 ± 0.05

^{3}H-Q_{10} (1',2'-[^{3}H]-coenzyme Q_{10}) (ref. 5)

Tissue	Uptake†		
	2 hr	24 hr	7 days
Brain	0.09 ± 0.01	0.06 ± 0.002	0.01 ± 0.001
Heart	0.51 ± 0.08	0.56 ± 0.02	0.23 ± 0.07
Lung	1.04 ± 0.18	1.92 ± 0.15	0.34 ± 0.13
Liver	1.04 ± 0.19	3.42 ± 0.22	2.20 ± 0.04
Kidney	0.53 ± 0.09	0.51 ± 0.10	0.09 ± 0.01
Blood‡	5.18	1.89	0.01

*) value : mean ± S.E. of 2 (5 min, 10 min) or 3 (20 min, 30 min) experiments (DAR)

†) dose : 0.6 mg/kg, value : mean ± S.E. of 3 experiments (μg/g wet tissue)

‡) Blood radioactivity declined in the two phases with half lives of 1.2 hr and 17.8 hr in rats. C_p = 2.15 exp (-0.555 t) + 4.83 exp (-0.039 t) (t : hr)

As HCO-60 forms a hard micell with lipophilic substances,the tissue distribution of [^{11}C]CoQ_{10}, in a short time, is considered to be affected by the nature of the emulsion. So, the further selection of a suitable emulsification method may be necessary for the development of [^{11}C]CoQ_{10} as a radiopharmaceutical.

ACKNOWLEDGMENT

The technical assistance in target handling and synthesis by Mr. M. Monma and Mr. M. Shinohara and the assistance in the animal experiments by Dr. H. Ohtomo and Mr. K. Kawashima are greatly acknowledged. The operation of the cyclotron by the members of Cyclotron and Radioisotope Center is also acknowledged.

REFERENCES

1. Crane F.L., Hatefi Y., Lester R.L. and Widmer C. - Biochim. Biophys. Acta 25 : 220 (1957)
2. Crane F.L., Widmer C., Lester R.L. and Hatefi Y. - Biochim. Biophys. Acta 31 : 476 (1959)
3. a. Hatefi Y., Haavik A.G., Fowler L.R. and Gliffiths D.E. - J. Biol. Chem. 237 : 2661 (1962)
   b. Ragan C.I. and Racker E. - J. Biol. Chem. 248 : 6876 (1973)
   c. Yamashita S. and Racker E. - J. Biol. Chem. 243 : 2446 (1968)
4. Mellors A. and Tappel A.L. - J. Biol. Chem. 241 : 4353 (1966)
5. Fujita T., Matsuura T., Takamatsu T., Tsutsumi J., Kinoshita K., Katayama K., Miyao K., Hamamura K., Kijima S., Shirato M. and Baba S. - Oyoyakuri 6(4) : 695 (1972)

6. Nakamura T., Sanma H., Himeno M. and Kato K. - Biomedical and Clinical Aspects of Coenzyme Q Vol.2 : 3 (1980)
7. Fujita T., Matsuura T., Takamatsu T., Hamamura K., Kijima S., Kinoshita K., Tsutsumi J., Katayama K., Miyao K., Baba S. and Shirato M. - Oyoyakuri 6(4) : 707 (1972)
8. Gloor U., Isler O., Morton R.A., Ruegg R. unt Wiss O. - Helv. Chim. Acta 41 : 2357 (1958)
9. Schunk C.H., Frickson R.E., Wong E.L. and Folkers K. - J. Am. Chem. Soc. 81 : 5000 (1959)
10. Iwata R., Takahashi T., Monma M. and Ido T. - J. Nucl. Med. 24 : p120 (1983)
11. Kloster G., Müller-platz C. and Laufer P. - J. Label. Compd. Radiopharm. 18 : 855 (1981)