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論　文

〔2－^{14}C, 5－^{3}H〕シトシンの合成

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Synthesis of 〔2－^{14}C, 5－^{3}H〕cytosine with use of Bromine and Tritium Gas.

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Abstract

A small scale synthesis of 〔2－^{14}C, 5－^{3}H〕cytosine was attempted by the bromination of 〔2－^{14}C〕cytosine followed by the catalytic exchange reaction with tritium gas. The radioactive materials were always used in the presence of those nonradioactive as carriers. Bromination were compared for the solutions of CCl_4 and CCl_4/H_2O. In the former, bromocytosine was formed without any byproduct, but the reaction rate was very slow. In the latter, the reaction occurred quite rapidly and bromocytosine was obtained in a relatively high yield (41～70 %), though undesirable bromouracil was also found. The double labelled cytosine was easily formed by a Br–^{3}H exchange between 〔2－^{14}C, 5－Br〕cytosine and $^{3}H_2$ gas using a catalyst of 10 % $Pd/CaCO_3$.

This paper also presents the experimental results for the adsorption of unreacted $^{3}H_2$ gas by active charcoal at 77 K. The amount of $^{3}H_2$ gas remaining in the reaction vessel was determined with a tritium monitor. The radioactivity was 13 μCi, which was 1/4000 that of the $^{3}H_2$ gas initially used.

Introduction

In the developement of the nuclear fusion reactor tritium brings about many chemical problems, especially in biological interests. We are planning to study the

fate of cytosine, one of the nucleic acid bases, associated with β-decay, when cytosine incorporates tritium inside the molecule.

Double labelled cytosine containing ^{14}C as a marker besides ^{3}H is synthesized by bromination of ^{14}C-cytosine[1-5], followed by a catalytic Br-^{3}H exchange[6-11]. The process is written as follows.

$$[2\text{-}^{14}C]Cy + Br_2 \longrightarrow [2\text{-}^{14}C,\ 5\text{-}Br]Cy + HBr \quad (1)$$

$$[2\text{-}^{14}C,\ 5\text{-}Br]Cy + {}^{3}H_2 \xrightarrow{\text{catalyst}} [2\text{-}^{14}C,\ 5\text{-}^{3}H]Cy + HBr \quad (2)$$

The purpose of the present study is to synthesize the double labelled cytosine with high yield in a small scale.

Some informations about the adsorption of unreacted tritium gas by active charcoal is also described in relation to tritium safety handling.

Experimental

1. Materials

[2-^{14}C]cytosine (specific activity=56 mCi/mmol or 2.07 GBq/mmol) was obtained from Moravek Biochemicals (California) and $^{3}H_2$ gas, from New England Nuclear (Massachusetts). Other reagents and solvents (Wako Chemicals) were of analytical grade.

2. Synthesis of [2-^{14}C, 5-Br]cytosine

The bromination in CCl_4 was compared with that in CCl_4 and water, in advance, by using nonlabelled cytosine. Cytosine and bromine in 1 ml of CCl_4 ($Cy : Br_2 = 1:1$) were placed in a sealed ampoule (1.5 ml) with a magnetic stirrer, and allowed to react under stirring at 85 °C for 10 h. On the other hand, the mixture of cytosine dissolved in 1 ml of water and bromine in 0.1 ml of CCl_4 ($Cy : Br_2 = 1 : 1$) was similarly treated at 25 or 0 °C for 1 h.

The bromination of ^{14}C-cytosine was performed in the system of CCl_4/water at 0 °C for 1 h. 4.0 μCi (148 KBq, 8.0 μg) of [2-^{14}C]cytosine was used with 0.9 mg of nonlabelled cytosine as a carrier.

3. Synthesis of [2-^{14}C, 5-^{3}H]cytosine[9]

The [2-^{14}C, 5-Br]cytosine synthesized above, 3 mg of 10 % palladium on calcium carbonate catalyst, and 150 μl of 0.3 N NaOH were placed in a reaction vessel. Two break-seal ampoules containing $^{3}H_2$ gas (48 mCi or 1.78 GBq, 0.018 ml (STP)) and H_2 gas (0.37 ml (STP)), respectively, were attached to the vessel ($BrCy : {}^{3}H_2 + H_2 = 1:2$), as shown in Fig.1. The total volume of the vessel was 7.6 ml. The alkaline condition

was used to minimize the exchange reaction between $^{3}H_2$ gas and water[11]. After opening the break-seal tips-1 and-2, the reaction was carried out at 80 ℃ for 1 h.

And then, excess $^{3}H_2$ and H_2 gases in the reaction vessel were adsorbed by active charcoal at 77 K, as described later. The catalyst was separated by centrifugation. The supernatant solution containing tritium water (5.7 μci or 211 KBq) produced by the exchange reaction was pipetted off and subsequently evaporated to dryness. Labile tritium in the products was removed by repetition of dissolution in water and subsequent evaporation.

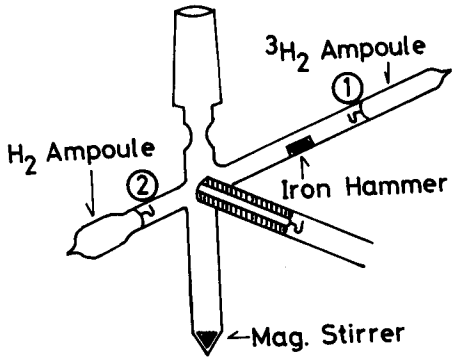

Fig. 1. Reaction vessel for catalytic Br-^{3}H (or H) exchange.
①,②: break-seal tips.

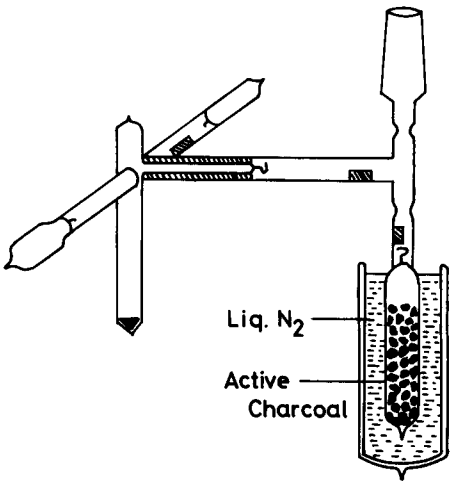

Fig. 2. Apparatus for adsorption of tritium and hydrogen gas by active charcoal.

4. Adsorption of $^{3}H_2$ and H_2 gases on active charcoal

The reaction vessel was connected to a break-seal tube (4.9 ml) containing 0.9 g of granular active charcoal (Tsurumi-HC-30F) degassed at 400 ℃ for 1 h, as shown in Fig.2. $^{3}H_2$ and H_2 gases were adsorbed on the charcoal at 77 k for 15 min.

The radioactivity of the $^{3}H_2$ gas still remaining in the reaction vessel was measured as follows. The vessel was broken in a polyethylene bag having the capacity of 45 1. The whole gas in the bag was let into a tritium monitor (Aloka, MGR-108R, Japan, pumping speed 7.5 1/min), and the activity was recorded (Fig. 3). After the bag was deflated, it was again inflated with fresh air. This process was repeted twelve times. The integrated activity was 13 μCi (481 KBq), which was 1/4000 of $^{3}H_2$ gas initially used, as shown in Fig. 4.

5. Analysis of products

The products obtained in the experiment on nonradioactive cytosine were ana-

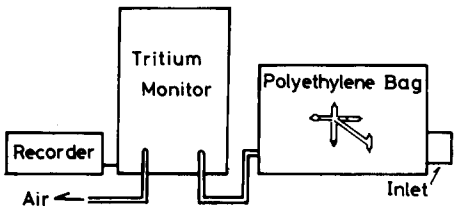

Fig. 3. Tritium-monitor and recording instrument.

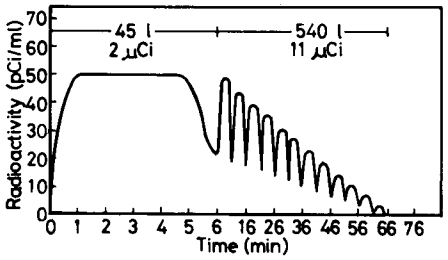

Fig. 4. Tritium activity change exhausted from the reaction vessel.

lyzed with a paper chromatograph (support: Toyo filter paper No. 50, developing solvent: n-butanol/water (86/14), amount of sample: 10 μl (~10 μg of Cytosine), developing time: 4 h). The chromatogram spots developed on paper was quantitatively measured using a dual-wavelength chromato-scanner (CS-900, Shimazu, λ_S: 290 nm, λ_R: 350 nm). The R_f values of cytosine, boromocytosine, uracil and bromouracil were 0.2, 0.4, 0.4 and 0.6 respectively.

It was noted that bromocytosine and uracil showed the same R_f value. Radioactive products were, therefore, analyzed with an HPLC (Model 520, Gasukuro kogyo Co. Ltd., Column: unisil pack Q C18, particle size: 5 μm, 4.6 $\phi \times 250$ mm, mobile phase: 10^{-4} M NaH_2PO_4 solution, pressurre: 100 kg/cm^2, flow-rate: 0.5 ml/min). Eluate, monitored by a UV-detector (254 nm), was fractionated into scintillation vials (28 mmϕ, polyethylene) by every 0.2 ml using a fraction collector (LKB 2111 Multi Rac, Sweden).

Being mixed with 12.5 ml of toluene-based scintillation counting solution and 2.5 ml of ethanol, each HPLC fraction was subjected to the measurement of ^{14}C- and ^{3}H-radioactivities with a liquid scintillation counter (Packard, Tri-carb 460CD). The results were analyzed by a calculator and a plotter (Hewlett-Packard 9815A and 9862A).

Results and Discussion

The paper chromatograms of the products obtained in the bromination of cytosine are shown in Fig. 5. The chromatogram-A is a result in the CCl_4 system and -B, in the CCl_4/H_2O system. The formation of bromouracil is recognized in the latter. The yields are shown in Table 1. In CCl_4, the yield of bromocytosine was~25 % at 85 °C for 10 h. A large amout of unreacted cytosine was found. In CCl_4/water, yellowish

color disappered very rapidly even at room temperature and 0 °C. Most of cytosine disappeared and bromcytosine formed in relatively high yield (41~70 %). Undesirable bromouracil was also produced by 12 ~18 %. The difficulty of accurate addition of bromine is supposed to be responsible for the scattering of the distribution.

The results of bromination of [2-^{14}C]-cytosine are shown in Fig. 6-A and Table I. Fig. 6-A shows HPLC chromatograms observed by UV absorption and by radioactivity for the brominated [2-^{14}C]cytosine solution. The percentage distributions calulated from the both results agree well with each other and are similar to that examined with nonradioactive cytosine. Recovery of radioactive ^{14}C-products,

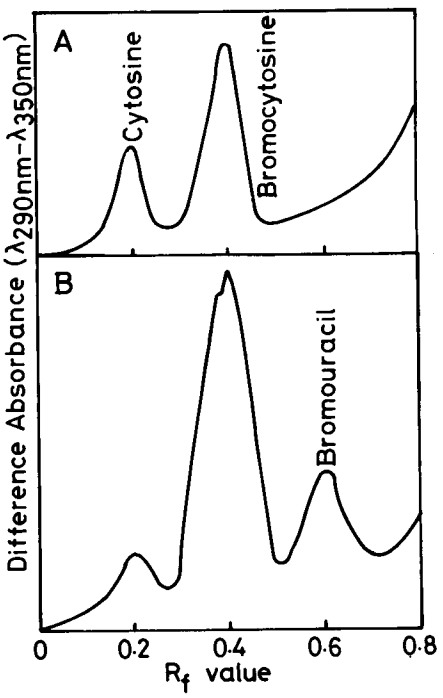

Fig. 5. Paper chromatograms of brominated cytosine solutions in CCl_4 (A) and CCl_4/H_2O (B).

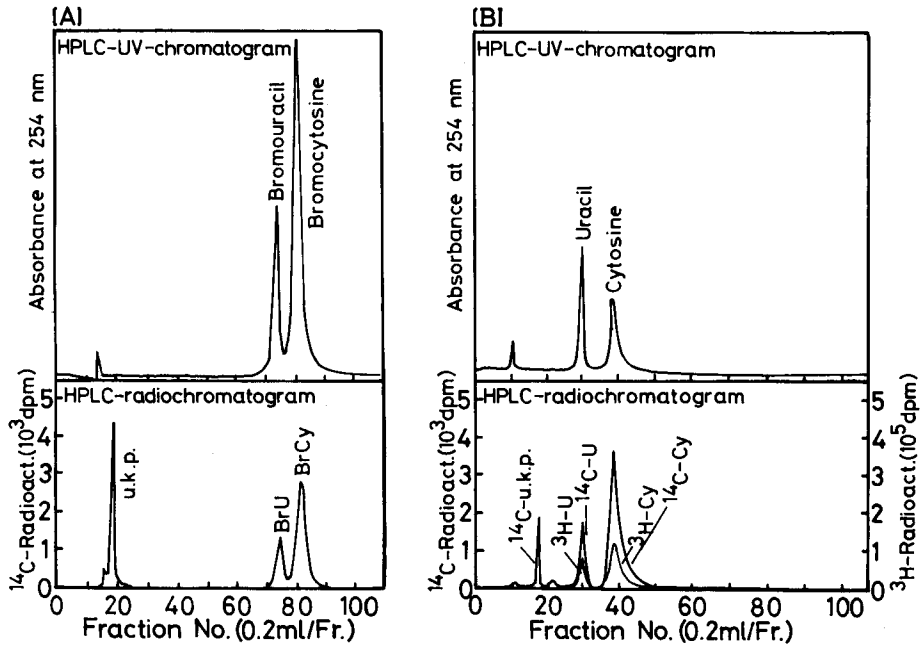

Fig. 6. HPLC-UV-chromatograms and HPLC-radiochromatograms. A : bromination of [2-^{14}C]cytosine, B : Br-^{3}H (or H) exchange reaction in [2-^{14}C , 5-Br]cytosine.

including unknown one, was 90 % (3.6 μCi or 133 KBq).

Table I. Products distribution in bromination of cytosine or [2-^{14}C]cytosine.

Run	Cy mg	Br_2 µl	Solvent	Temp °C	Time h	Analysis		Distribution, % Cy	BrCy	BrU	uncapt. or u.k.p.
1 - 1	1	0.48	CCl_4	85	10	P.C.	UV	35	26	0	(39)
1 - 2	0.5	0.24	CCl_4	85	10	P.C.	UV	20	23	0	(57)
1 - 3	1	0.48	CCl_4+H_2O	25	1	P.C.	UV	10	70	12	(8)
1 - 4	1	0.48	CCl_4+H_2O	0	1	P.C.	UV	0	41	12	(47)
1 - 5	1	0.48	CCl_4+H_2O	0	1	HPLC	UV	17	67	5	(10)
1 - 6*	0.9	0.44	CCl_4+H_2O	0	1	HPLC	UV	0	49	14	(37)
							^{14}C	0	54	18	24

* experiment with the use of [2-^{14}C]cytosine. ( ) calculated values.

Table II. Products distribution in Br-^{3}H(H) exchange reaction between [2-^{14}C,5-Br]cytosine and $^{3}H_2/H_2$ mixed gas.

Run	^{14}C,Br-Cy mg	$^{3}H_2/H_2$ ml	Temp °C	Time h	Analysis		Distribution, % BrCy	BrU	Cy	U	uncapt. or u.k.p.
2 - 1*	(1.23)	0.38	80	1	HPLC	UV	0	0	58	5	(37)
2 - 2**	(1.00)	0.38	80	1	HPLC	UV	0	0	47	15	(38)
						^{14}C	0	0	51	18	23
						^{3}H	0	0	81	17	0

* experiment continued from the Run No. 1-5 ; hydrogen gas was used .

** experiment continued from the Run No. 1-6.

( ) calculated values.

The results of the Br-^{3}H catalytic exchange are shown in Fig. 6-B and Table II. Bromocytosine disappeared completely and the corresponding amount of cytosine was obtained. Bromouracil did in a similar way. It is interesting that an unknown peak, which was not detected by UV absorption, was observed in the radiochromatogram of ^{14}C. The percentage is nearly in accordance with that uncaptured by UV absorption. The recovery of ^{14}C-labelled products including the unknown one was 58 % (2.1 μCi or 77 KBq).

The percentage yield of the double labelled cytosine determined by ^{3}H activity was larger than by ^{14}C activity. Total activity of tritium in cytosine and uracil molecules is only 348 μCi (12.9 MBq), which is 3 % of the radioactivity expected from the

amounts of $^{3}H_2$ and H_2 gases used. However, a sufficient amount of [2-^{14}C, 5-^{3}H]-cytosine can be synthesized by the method described in the present study, using the carrier free radioactive materials.

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