RAPID SYNTHESIS OF ^{14}C FORSKOLIN BY A ROUTE SUITABLE FOR ^{11}C LABELING

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SUMMARY

Radiolabeled forskolin was synthesized by acetylation of deacetylforskolin with ^{14}C-labeled acetic acid in the presence of dicyclohexylcarbodiimide. Separation of labeled forskolin of greater than 97% radiochemical purity was achieved by HPLC with a reversed-phase column. The synthesis and purification of labeled forskolin were achieved in less than 30 min and the radiochemical yield of labeled forskolin after HPLC purification was about 30%. These methods are therefore applicable for production of ^{11}C-forskolin which may be useful for PET second messenger imaging.

Key Words: Radiolabeled Forskolin, Dicyclohexylcarbodiimide, Second Messenger Imaging Agent

INTRODUCTION

Direct investigation of neuronal function by nuclear medicine techniques has so far been confined to studies on the biosynthesis of neurotransmitters or binding ability to receptors using radiolabeled neurotransmitters or their analogs. However, the cellular response is not necessarily proportional to the amount of neurotransmitter bound to the receptor. Local rates of blood flow or glucose metabolism represent overall removal

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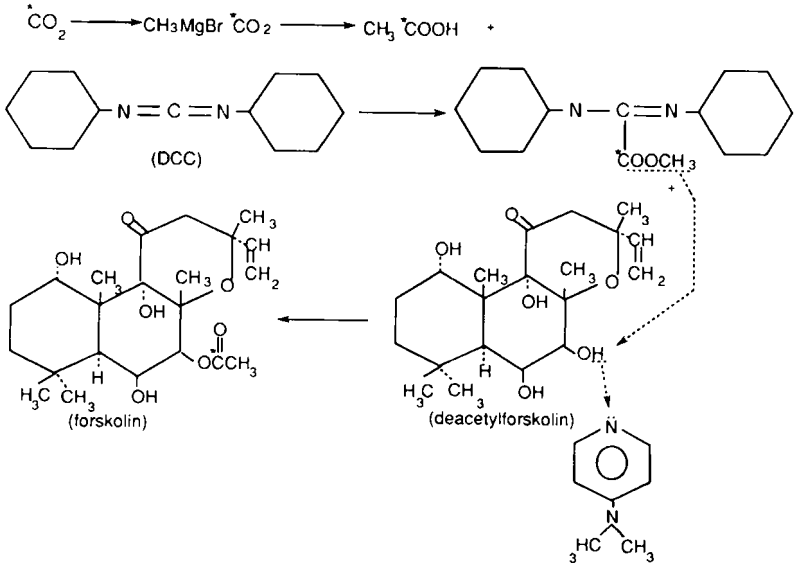
activity and do not give information about specific pathways. Tracers which reveal more direct effects of neurotransmitters action are desirable. Neurotransmitters binding causes stimulation of second messenger systems such as those involving cyclic AMP or phosphatidylinositol (PI)(1). We have reported the enzymatic synthesis of radiolabeled inositol as an imaging agent for the PI related second messenger system (2). Several neurotransmitters and hormones are mediated by an adenylate cyclase (AC) related second messenger system (3-7). It has been reported that forskolin, a diterpene isolated from Coleus forskolii (8), has specific binding and activating abilities for AC (9). Tritium-labeled forskolin has been utilized as a tool for in vitro AC-mapping of brain slices etc. (10-11). We have planned to synthesize ^{11}C-labeled forskolin from deacetylforskolin and ^{11}C-labeled acetic acid (Scheme 1), as a tool to map AC activity quantitatively in vivo using positron emission tomography. As a preliminary study for the synthesis of ^{11}C-forskolin, we attempted to synthesize ^{14}C-forskolin from ^{14}C-acetic acid and deacetylforskolin using dicyclohexylcarbodiimide (DCC)(12).

Scheme 1. Synthesis of radiolabeled forskolin



EXPERIMENTAL

MATERIALS

Forskolin, deacetylforskolin, DCC and dimethylaminopyridine were obtained from Sigma Chemical Co.. Other reagents of analytical grade were from Wako Chemical Co., Ltd. ^{14}C-Acetic acid sodium salt ($CH_3{}^*COONa$; 2 GBq/mmol) was obtained from Amersham Japan Co., Ltd..

SYNTHESIS OF ^{14}C-FORSKOLIN

^{14}C-Acetic acid sodium salt (3.7 MBq, 1.85 umol)/50 µL H_2O was mixed with 50 µL of 85 % H_3PO_4, and ^{14}C-acetic acid was extracted into 3 ml of ether in an automatic shaker. The ether layer was dehydrated with P_2O_5 (0.2 g), and its aliquot (25 µL) was transferred to a reaction tube and incubated with 100 nmol of deacetylforskolin, 200 nmol of DCC, 100 nmol of dimethylaminopyridine and 25 µL of CH_2Cl_2 at 60 °C for 10 min. The effect of the amount of acetic acid carrier on the radiochemical yield of ^{14}C-forskolin in the above reaction was judged by radio-TLC scanner. Labeled forskolin was purified by high-performance liquid chromatography (HPLC) on a reversed-phase column (Nihon Bunko Co. Ltd., Finepak $SILC_{18}$-5, 7.6 mm x 250 mm), and eluted with acetonitrile/H_2O (40:60), at 1 mL/min.

IDENTIFICATION OF SYNTHESIZED FORSKOLIN

Identification of synthesized product was checked by TLC (hexane:ethyl acetate=65:35), HPLC, and measurement of melting point (from ethyl acetate/petroleum ether; 230-232 ℃)(13).

MEASUREMENT OF CHEMICAL AND RADIOCHEMICAL YIELD

Chemical and radiochemical yields of forskolin were determined by TLC. The TLC plate was stained with anisaldehyde and spots were quantitated by densitometry (Shimadzu CS-9000). The radiochemical yield was calculated from the counts determined by

a TLC scanner (Packard, Model 7230), with ^{14}C-acetic acid sodium salt as a standard.

RESULTS AND DISCUSSION

Deacetylforskolin (7-deacetylforskolin), used as a precursor for the synthesis of forskolin, has four hydroxyl groups (1-, 6-,7- and 9-OH) capable of being acylated. However, Bhat et al. (14) demonstrated that the 7-OH group in 7-deacetylforskolin was preferentially acylated with propionic anhydride and pyridine under controlled conditions to give the 7-propionate. Any other OH group in forskolin is acetylated only after the acetylation of the 7-OH. The 6-OH group, for example, is acetylated by heating at reflux for 90 h to give the 1,6-diacetate in low yield, and the 9-OH group has not been observed. We tried to acetylate the 7-OH group of deacetylforskolin with radiolabeled acetic acid. The synthesized product exhibited the same Rf value in TLC and retention time in HPLC as the standard forskolin (TLC, Rf of 0.59; HPLC, retention time of 11.9 min). The Rf values of acetylforskolin, which was obtained from further acetylation of forskolin with DCC at 100 °C for 30 min and deacetylforskolin, were 0.73 and 0.45, respectively. Moreover, its melting point (224-227 °C) was also consistent with the standard forskolin. This melting point is greatly different from those of deacetylforskolin (176-178 °C), 1-acetylforskolin (203-206 °C) and 1,6-acetylforskolin (216-218 °C)(13). Therefore, the synthesized product was confirmed to be forskolin.

On the basis of preliminary experiment with unlabeled material, the optimum ratio of acetic acid, DCC and dimethylaminopyridine to the deacetylforskolin was concluded to be 1:2:1:1. Table I shows the effect of amount of acetic acid, DCC and dimethylaminopyridine on the yield of forskolin when the content of deacetylforskolin was fixed at 100 nmol and the ratio of the first three reagents was fixed at 1:2:1. The result

Table I. Effect of various amounts of acetic acid, dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine on the yield of forskolin from 100 nmol of deacetylforskolin.

Acetic acid	DCC	Dimethylamino-pyridine	Yield of forskolin
nmol	nmol	nmol	%
100	200	100	29.8
500	1000	500	54.2
1000	2000	1000	61.5
2000	4000	2000	77.9

indicates that high synthetic yields of forskolin could be achieved by increasing the amounts of acetic acid, DCC and dimethylaminopyridine relative to deacetylforskolin.

DCC forms dicyclohexylurea in the presence of water. Accordingly, for the synthesis of radio-labeled forskolin, we extracted ^{14}C-acetic acid into ether from an aqueous solution of the sodium salt acidified with 85% H_3PO_4, and dehydrated it by P_2O_5. The extraction efficiency of ^{14}C-acetic acid into the ether layer was 91% and the recovery after dehydration treatment was greater than 98%. Fig. 1 shows the effect of the amount of the added acetic acid carrier on the radiochemical yield of ^{14}C-forskolin. The radiochemical yield increased with the amount of acetic acid up to a ratio of 2:1 with respect to deacetylforskolin, but decreased at higher ratio.

The purification of ^{14}C-forskolin was carried out by HPLC . A typical elution pattern is shown in Fig. 2A. Retention times for ^{14}C-acetic acid, deacetylforskolin, DCC-^{14}C-acetic acid complex and ^{14}C-forskolin were 1.9, 6.7, 9.5 and 11.9 min, respectively. The ^{14}C-forskolin fraction was recovered and rechromatographed on the same column (Fig. 2B). The radiochemical purity of ^{14}C-forskolin reached more than 97%. Measured from the start of the ^{14}C-acetic acid extraction, the total synthesis time including HPLC purification was 30-35 min and the radiochemical

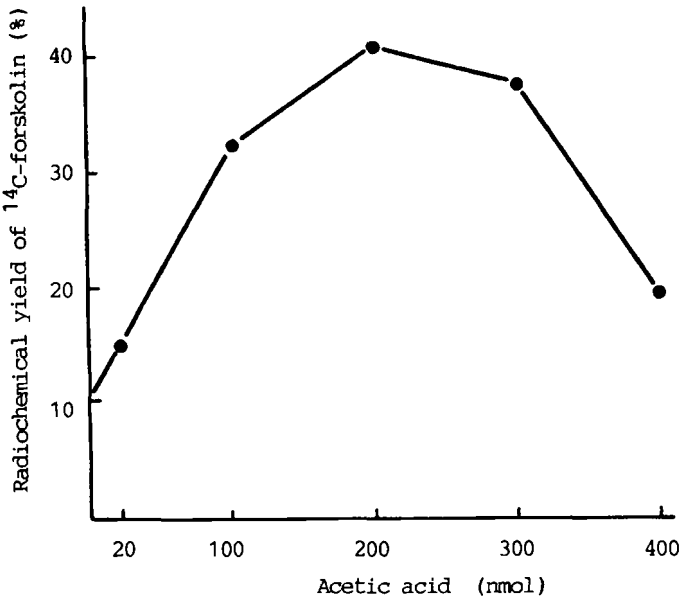
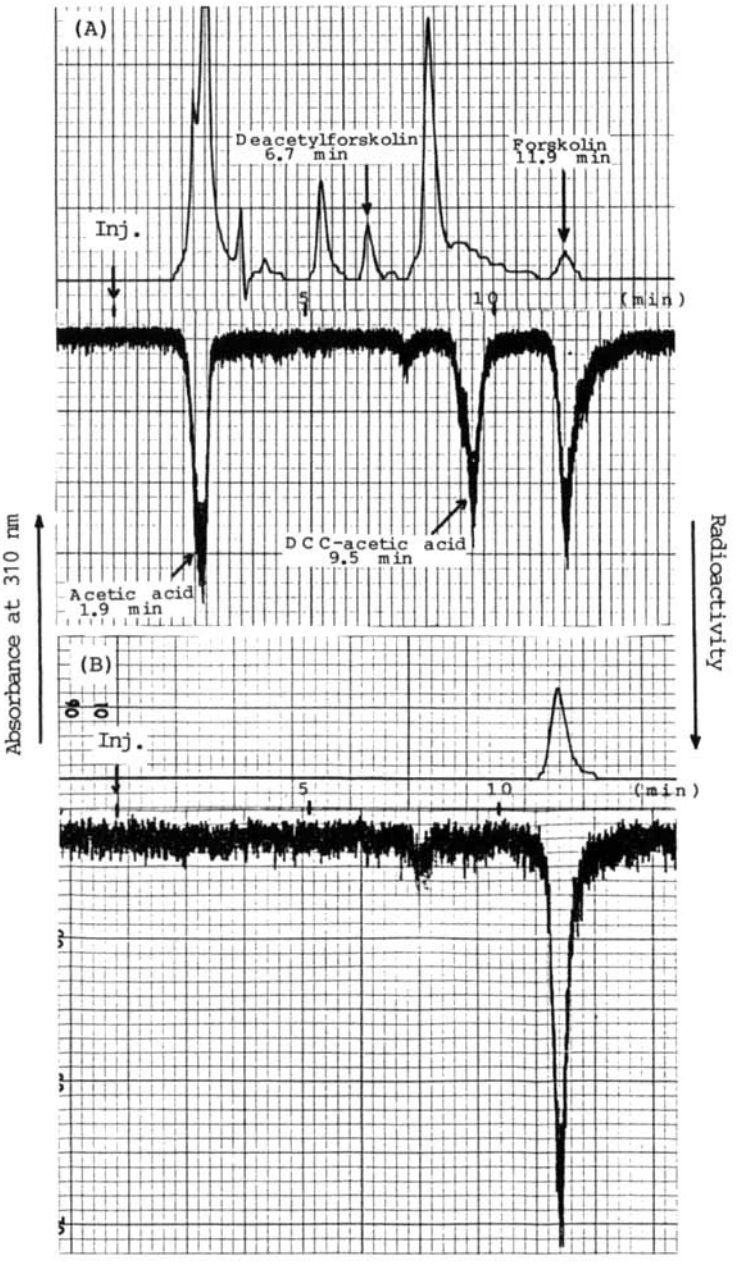


Figure 1. Effect of various amounts of acetic acid on the radiochemical yield of ^{14}C-forskolin. The reaction mixture contained 27.8kBq/ 18.9nmol of ^{14}C-acetic acid in 25 µL of ether and 100 nmol of deacetylforskolin, 200 nmol of dicyclohexylcarbodiimide, 100 nmol of dimethylaminopyridine and 20-400 nmol of acetic acid in 25 µL of CH_2Cl_2.

yield was about 30%. If this method is to be applied to the synthesis of ^{11}C-forskolin, it will be necessary to also consider the yield of ^{11}C-acetic acid(15).

The correlation between the structure and the activating ability of AC has been examined using forskolin and its derivatives for AC obtained from brain and cardiac cell membrane (13,16). These reports indicates the critical nature of the 9-OH group or of the concomitant presence of the 1- and 9-OH groups for pharmacological activity. Our simple acylation method using the DCC reaction would be able to provide not only the 7-acetyl but also 7-propionyl, 7-benzoyl and other derivatives with binding ability for AC. Various neurotransmitter-receptors (β-adrenergic, etc.) are directly coupled to AC through the stimulatory guanyl nucleotide-binding protein (3-7). These



Retention time (min)

Figure 2. HPLC profile of the reaction mixture. (A); crude reaction mixture, (B); ^{14}C-forskolin purified by HPLC.

receptors can both activate AC activity and bring about accumulation of cyclic AMP in brain slices or synaptoneurosomes. Radiolabeled forskolin, synthesized by our simple DCC method, may be a useful AC-mediated second messenger imaging agent for brain or cardiac muscle.

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