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Journal of Chromatography A, 921 (2001) 331–334

JOURNAL OF CHROMATOGRAPHY A

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Short communication

Separation of radioactive metabolites in cultured tea cells fed with [^{14}C]phenylalanine using high-speed counter-current chromatography

Qizhen Du[a], Hao Cheng[b], Yoichiro Ito[c,*]

[a]*Institute of Food and Biological Engineering, Hangzhou University of Commerce, Hangzhou 310035, China*
[b]*Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou 31008, China*
[c]*Laboratory of Biophysical Chemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Bldg. 10, Rm 7N322, 10 Center Drive MSC 1676, Bethesda, MD 20892-1676, USA*

Received 9 February 2001; received in revised form 10 April 2001; accepted 10 April 2001

Abstract

Separation of radioactive metabolites in cultured tea cells fed with [^{14}C]phenylalanine was conducted using high-speed counter-current chromatography. Among seven components obtained our studies focused two metabolites, i.e. (−)-epicatechin and D,L-catechin. The specific radioactivity of (−)-epicatechin was 212.01 KBq/mg, amounting to 8.5% of the total radioactivity of ethyl acetate extract while that of D,L-catechin was 1.0006 MBq/mg or 5.4% of the total.

Keywords: Counter-current chromatography; Tea; Catechins; Polyphenols

1. Introduction

Catechins are the main bioactive components in green tea, which show antioxidant activity, anti-mutagenicity, and growth inhibition of cancer cells [1–3]. However, the metabolic pathway of catechins in animals has not been reported. Preparation of labeled catechins is a key step in such investigation. Cheng [4] has shown that [^{14}C]D,L-catechin and [^{14}C](−)-epicatechin in cultured tea cells fed with [^{14}C]phenylalanine. Catechins are polyphenol compounds which tends to be irreversibly adsorbed onto the solid support in normal column chromatography. Being a support-free chromatography, high-speed counter-current chromatography (HSCCC) can efficiently separate catechins with an excellent recovery [4–7]. In this paper, we processed the ethyl acetate extract of cultured cells from tea plant by HSCCC to isolate [^{14}C]D,L-catechin and [^{14}C](−)-epicatechin.

2. Experimental

2.1. Apparatus

The HSCCC instrument used in the present study

*Corresponding author. Tel.: +1-301-496-1210; fax: +1-301-402-3404.
E-mail address: itoy@nhlbi.nih.gov (Y. Ito).

0021-9673/01/$ – see front matter
PII: S0021-9673(01)00862-7

was a Model GS-10A multilayer coil planet centrifuge fabricated by Beijing Institute of New Technology Application, Beijing, China. It was equipped with a 230-ml capacity multilayer coil of 1.6 mm I.D. PTFE (polytetrafluoroethylene) tubing. The separation was performed at 800 rpm. The mobile phase was delivered with a Waters 510 HPLC pump (Millipore, Milford, MA, USA). An injection loop was used for sample loading and a UV detector (8823A, Beijing, China) for monitoring the effluent.

2.2. Reagents

Hexane and ethyl acetate were of an analytical grade and purchased from Shanghai Chemical, Shanghai, China. D,L-catechin and (−)-epicatechin were purchased from Sigma (St. Louis, MO, USA)

2.3. Preparation of cultured tea cells

2.3.1. Extraction of metabolites from cultured tea cells

The cultured tea cells fed with [^{14}C]phenylalanine were levigated at 0°C followed by extraction with 80% ethanol. The extract was filtered and evaporated to dryness in vacuum at 25°C. The residues thus obtained were extracted with ethyl acetate which was again evaporated to dryness at 25°C. The residues were then lyophilized to obtain ethyl acetate extract powder.

2.4. HSCCC separation

The HSCCC separation was performed with a two-phase solvent system composed of hexane–ethyl acetate–water (10:1:12. v/v) using the upper organic phase as the mobile phase and the lower aqueous phase as the stationary phase. In each experiment the column was entirely filled with the stationary phase followed by injection of sample solution containing 100 mg of the ethyl acetate extract in 10 ml of the mobile phase through the sample loop. Then the column was rotated at 800 rpm and eluted with the mobile phase at 1.5 ml/min. The effluent from the column outlet was continuously monitored at 280 nm with a UV detector (8823A, Beijing Institute of New Technology Application, Beijing, China), and fractioned into test tubes using a fraction collector.

2.5. Determination of radioactivity

An aliquot (0.5 ml) of the crude extract and HSCCC fractions was each mixed with 10 ml of scintillant [2,5-diphenyloxazole (PPO)–1.4-bis(5-phenyloxazoly1-2)benzene] (POPOP)–dimethylbenzene–oxitaol, 0.5 g:5 g:650 ml:150 ml, and their radioactivity was determined with a Tri-carb 1900 Packard liquid scintillation counter.

2.6. HPLC analysis

The crude sample and HSCCC fractions were analyzed by HPLC as follows: a BDS column (5 μm, 250×4.6 mm) (Elite, Dalian, China) was used. The mobile phase was a linear gradient of solvents A (acetonitrile) and B (2% aqueous acetic acid) starting at A–B (8:92, v/v) and ending at A–B (85:15, v/v) for 23 min at a flow-rate of 1.4 ml/min.

3. Results and discussion

3.1. HSCCC separation

Fig. 1 shows HPLC analysis of the crude ethyl acetate extract of tea cells fed with [^{14}C]phenylalanine monitored by absorbance at 280 nm. It shows that D,L-catechin (D,L-C) and (−)-epicatechin (EC) are two major metabolites extracted with ethyl acetate. The HSCCC separation of the same sample is illustrated in Fig. 2. Seven peaks

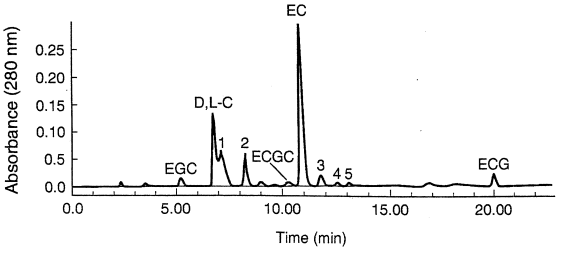

Fig. 1. HPLC analysis of ethyl acetate extract of cultured tea cells fed with [^{14}C]phenylalanine. Experimental conditions: sample size: 10 μl for each separation; BDS column (5 μm, 250×4.6 mm; Elite); elution: a linear gradient of solvent A (acetonitrile) and solvent B (2% aqueous acetic acid) starting at A–B (8:92, v/v) and ending at at A–B (85:15, v/v) at a flow-rate of l.4 ml/min.

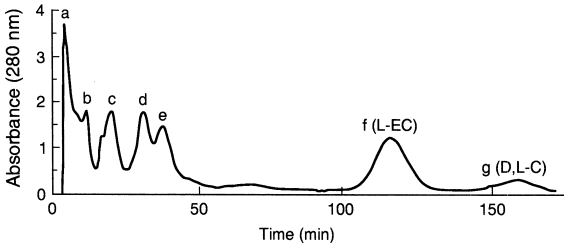

Fig. 2. HSCCC separation of ethyl acetate extract of cultured tea cells fed with [^{14}C]phenylalanine. Experimental conditions: apparatus: type-J HSCCC centrifuge equipped with a multilayer coil of 1.6 mm I.D. and 230 ml capacity; sample: 100 mg of ethyl acetate extract of cultured tea cells in 10 ml of mobile phase; solvent system: hexane–ethyl acetate–water (10:1:12, v/v); sample size: 10 ml; mobile phase: lower aqueous phase; flow-rate: 1.5 ml/min; revolution: 800 rpm.

were produced. Peak a contained a mixture of colored compounds with unknown structure. Peak b–e, all containing unknown compounds, correspond to peaks 5, 2, 3 and 1 in the HPLC chromatogram, respectively. Fractions corresponding to peaks f and g in HSCCC separation contained (−)-epicatechin and D,L-catechin, respectively, as identified by HPLC with their standard samples as illustrated in Fig. 3.

3.2. Radioactivity analysis of labeled compounds

Table 1 summarizes the results of radioactivity analyses of peak fractions obtained by HSCCC (Fig. 2). The radioactivity of peaks a and b was 20.7% of the total radioactivity of the ethyl acetate extract sample, and peak c, 56%, peak d, 38%, peak e, 4.8%,

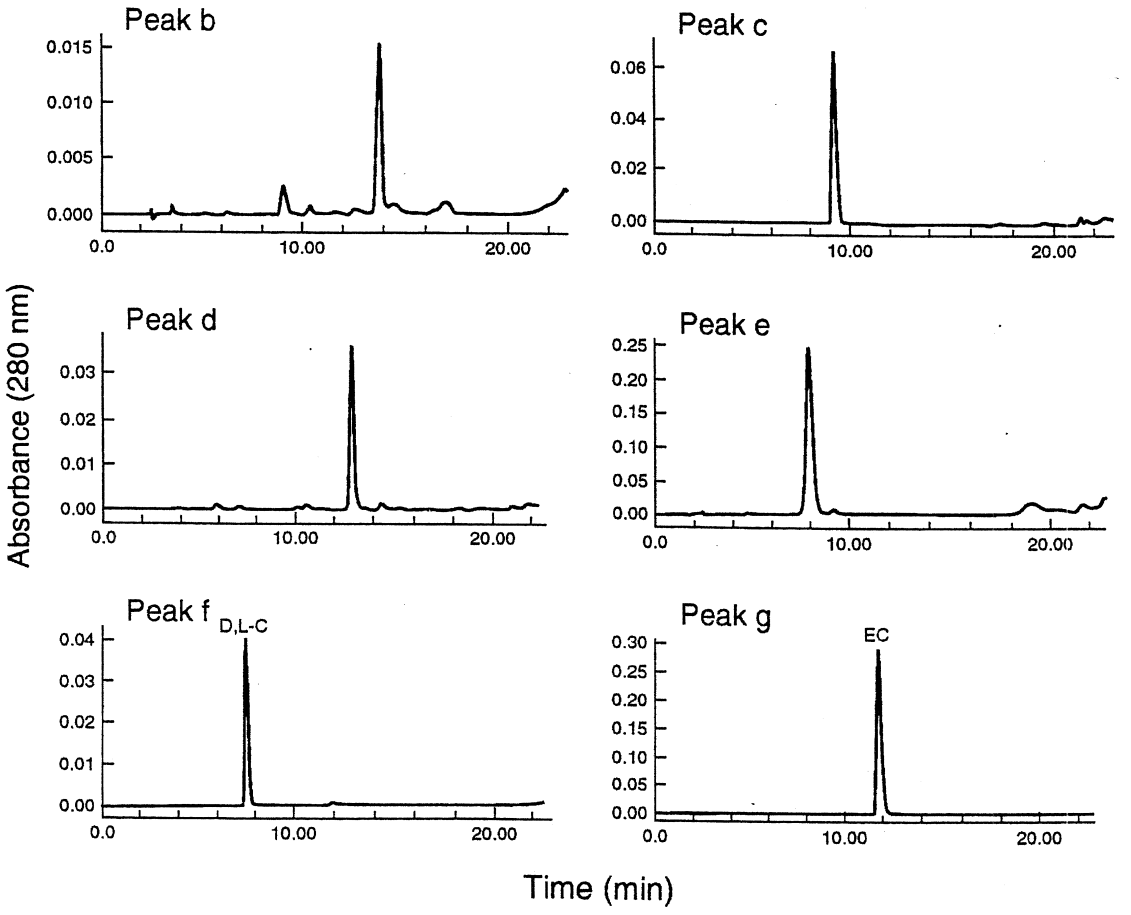

Fig. 3. HPLC analyses of peak fractions obtained by HSCCC. Experimental conditions as in Fig. 1.

Table 1
Radioactivity distribution of HSCCC fractions corresponding to peaks a–g

Component	Radioactivity (KBq)	Radioactivity ratio in EtOAc extract (%)
Peaks a and b	2037.99	20.5
Peak c	5559.44	56.0
Peak d	376.292	2.8
Peak e	480.483	4.8
Peak f (EC)	841.075	8.5
Peak g (D,L-C)	534.992	5.4
Stationary phase in the column	0	0

peak f, 4.5% and peak g, 5.4% as shown in the table. Specific radioactivity of peaks f and g was 212.01 KBq and 1.006 MBq, respectively. Since the compounds retained in the column with the stationary phase show no radioactivity, all specific activity was recovered in peaks a–g as shown in the table. The results clearly indicate that the ^{14}C-labeled phenylalanine was not incorporated into gallate-type catechins including (−)-epicatechin gallate (ECG) and (−)-epigallocatechin gallate (EGCG).

Since the HSCCC eliminates the use of solid support, the method yields a high recovery even for a minute amount of samples and no sample is lost onto the stationary phase. Therefore, it is especially suitable for the separation of radioisotope-labeled compounds. The results of the present experiment clearly show the advantages of HSCCC over other chromatographic method with the solid support.

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