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An automated sequential injection spectrophotometric method for evaluation of tyramine oxidase inhibitory activity of some flavonoids

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ABSTRACT

An automated sequential injection (SI) spectrophotometric system has been developed for evaluation of tyramine oxidase (TOD) inhibitory activity. The method is based on the inhibition of TOD that catalyzes the oxidation of tyramine substrate to produce aldehyde and hydrogen peroxide (H_2O_2). The produced H_2O_2 reacts with vanillic acid and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD) to form a quinoneimine dye, the absorbance of which is measured of absorbance at wavelength of 490 nm. The decrease of the quinoneimine dye is related to an increase of TOD inhibitory activity. Under the optimum conditions: 1.0 mM tyramine, 8 U mL^{-1} TOD, 1.0 mM vanillic acid, 1.0 mM 4-AA and delay time of 10 s, some flavonoid compounds were examined for the TOD inhibitory activity expressed as IC_{50} value. It was found that flavonols (quercetin and myricetin) and flavans (epicatechin gallate (ECG) and epigallocatechin (EGC)) showed higher TOD inhibitory activity than flavones and flavanones. The results of IC_{50} values obtained from the proposed method and a batch-wise method were not significantly different from each other. Moreover, the SI system enabled automation of the analysis, leading to more convenient, more sensitive and faster analysis than the batch-wise method. A precise timing of the system also improves precision and accuracy of the assay, especially when the measurement of absorbance at non-steady state condition is involved.

1. Introduction

Monoamine oxidase (flavin-containing; MAO; E.C. 1.4.3.4) is an enzyme that oxidizes various physiologically and pathologically important monoamine neurotransmitters and hormones. The reaction catalyzed by MAO is generally presented as

$$RCH_2NH_3^+ + O_2 + H_2O \rightarrow RCHO + NH_4^+ + H_2O_2$$

MAO is bound to the outer membrane of mitochondria in most cell types in the body. The enzyme was originally discovered by Mary Bemheim [1] in the liver and was named tyramine oxidase (TOD). It exists in two isoforms, namely MAO-A and MAO-B, which are the products of two distinct genes [2]. MAOs play a vital role in the inactivation of neurotransmitters and MAO dysfunction (too much or too little MAO activity) is related to a number of psychiatric and neurological disorders such as depression [3], schizophrenia [4], substance abuse, attention deficit disorder [5], migraines [6], and irregular sexual maturation. Monoamine oxidase inhibitors (MAOIs) can be used as drugs to regulate MAOs activities. The MAOI drugs are associated with numerous side effects that often limit their usefulness and tolerability. For example, the "cheese reaction" hypertensive crisis can occur when tyramine-rich foods are ingested in conjunction with MAOIs drugs [7,8]. In the presence of MAOIs, tyramine is not broken down and large amounts of it may get absorbed. High levels of tyramine can suddenly and dangerously elevate blood pressure.

Nowadays, substances derived from natural product are of interest because they usually have less side effects than synthesized drugs [9]. Flavonoid compounds have shown promising inhibitory activity on MAO [10–16]. In order to evaluate inhibitory activity of the potential substances, various methods have been developed for evaluation of MAO activity, as listed in Table 1. Different detection techniques such as colorimetry [17], spectrophotometry [17–22], capillary electrophoresis [23], bioluminescent [24], high performance liquid chromatography [25] and fluorometry [26–29] were employed. Spectrophotometry is often used in preference to the other techniques listed because the instrumentation is simpler and less expensive. It can be seen from Table 1 that the method based on the use of tyramine as a substrate and detection of H_2O_2 by forming

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Table 1
Various methods for assay of MAO inhibitory activity.

System	Substrate	Reagent	Product	Inhibitor/sample	Ref.
Colo. (450 nm)	Tyramine	2,4-Dinitrophenyl- hydrazine	2,4-Dinitrophenyl-hydrazone	–	[17]
Spec. (360 nm)	Kynuramine	–	Aldehyde	BAPN	[18]
Spec. (242, 280 nm)	Serotonin, benzylamine	Butyl acetate, cycrohaxan	Product in organic phase	Antler velvet	[19]
Spec. (498 nm)	Tyramine	Vanallic acid/4-AA/HRP	H_2O_2/quinoneimine dye	Clorgyline, pargyline	[20,21]
Spec. (490 nm)	Tyramine, benzylamine	Vanallic acid/4-AA/HRP	H_2O_2/quinoneimine dye	Clorgyline, pargyline	[22]
Spec. (490 nm)	Tyramine	Vanallic acid/4-AA/HRP	H_2O_2/quinoneimine dye	Quercetin	[10]
Flu. (Ex:315, Em: 380 nm)	Tyramine	Vanallic acid/4-AA/HRP	H_2O_2/Fluorescent	Poppy seedlings	[26]
Flu. (Ex:545, Em: 590 nm)	Tyramine	Amplexâ Red MAO assay kit	H_2O_2/Fluorescent	3-Heteroaryl-coumarin derivatives	[37]
Flu. (Ex:320, Em: 380 nm)	Kynuramine	–	4-Hydroxyquinoline	Tobacco	[28]
CE (280 nm)	Dopamine	–	Homovaniuic acid	Natural extract	[23]
Biolumi.	Aminopropylether	Methyl ester luciferin	Luminescence	Clorgyline	[24]
RP-HPLC	Kynuramine	–	4-hydroxyquinoline	β−carbolin alkaloids	[25]
SI-Spec. (490 nm)	Tyramine	Vanallic acid/4-AA/HRP	H_2O_2/quinoneimine dye	Flavonoids	This work

4-AA, 4-aminoantipyrine; BAPN, lathyrogen-β-aminopropionitrile; Biolumi, bioluminescent assay; CE, capillary electrophoresis; Colo, colorimetry; Flu, spectrofluorimetry; HRP, horseradish peroxidase; RP-HPLC, reversed-phase high performance liquid chromatography; SI, sequential injection; and Spec, spectrophotometry.

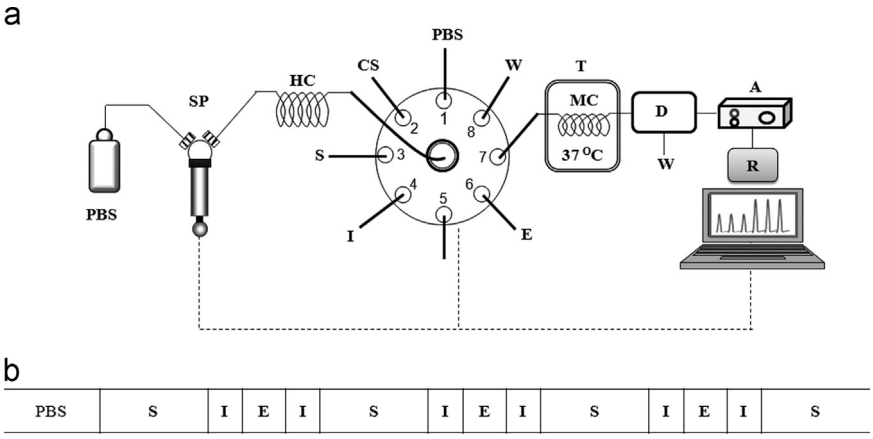

Fig. 1. (a) Manifold of sequential injection spectrophotometric system for evaluation of TOD-inhibitory activity; (b) sequence of solutions in a holding coil of the SI system; PBS: phosphate buffer solution, SP: syringe pump, HC: holding coil, E: TOD, S: tyramine, I: standard/sample (inhibitor), CS: chromogenic solution, MC: mixing coil, T: temperature controller, D: spectrophotometer, W: waste, A: home-made amplifier unit, and R: recorder.

a quinoneimine dye are widely used. However, when the assay is carried out manually, it is time consuming and susceptible to errors arising from inaccurate and imprecise timing of the various steps and final steady-state absorbance measurement.

Therefore, a sequential injection (SI) system was applied in this work in order to automate the assay of inhibitory activity on TOD. The same chemistry as is widely used in the batch-wise method was employed. It is based on measurement of the H_2O_2 formed during the oxidation of tyramine by the TOD, followed by reacting with vanillic acid and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD) to form a quinoneimine dye. The dye was then detected spectrophotometrically at wavelength of 490 nm. Some flavonoid compounds were identified for their IC_{50} values on TOD inhibition. Results obtained are compatible to those obtained by the batch-wise method [10]. Moreover, the SI system reduces analysis time and improves precision and accuracy of the assay due to its high degrees of automation.

2. Experimental

2.1. Chemicals

Tyramine oxidase (EC 1.4.3.4, 4.6 U mg^{-1} solid), quercetin, morin, and catechin were purchased from Tokyo Chemical Industry Ltd. (Japan). 4-Aminoantipyrine, peroxidase (POD), naringenin, and epigallocatechin were purchased from Sigma Chemical Co. Ltd. (USA). Tyramine was purchased from Nacalai Tesque Inc. (Japan). Other chemicals (NaOH, $NaH_2PO_4 \cdot 2H_2O$, Na_2HPO_4, ethanol, vanillic acid, kaempferol, luteolin, apigenin, and myricetin) were purchased from Wako Pure Chemical Industries Ltd. (Japan). A Milli-Q water obtained from the water purification system (Millipore, Tokyo, Japan) was used to prepare solutions.

2.2. Instrument setup

The manifold of the sequential injection (SI) system employed in the present study was schematically depicted in Fig. 1(a). The system was produced by MGC JAPAN Co., Ltd., Japan. It consisted of a syringe pump, a multiposition valve, a temperature controller and a spectrophotometric detector (UV–vis detector S-3702, SOMA OPTICS, Co., Ltd., Japan). PTFE tube (i.d. 0.8 mm, o.d. 1.58 mm) was used for making a holding coil (HC) and a mixing coil (MC), and connecting various devices of the system. An output signal from the spectrophotometer was amplified by a home-made amplifier unit before being recorded by a recorder. The SIA system was controlled by a computer via a SI controller software (MGC JAPAN Co., Ltd. Japan).

2.3. Preparation of solutions

Phosphate buffer solution (PBS) of pH 7.6 (0.2 M total phosphate) was prepared from NaH_2PO_4–Na_2HPO_4 by dissolving them in water. A stock solution of 1.0 mM tyramine was prepared by