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Fiber-optic in situ analysis of the catalytic kinetics of the alliin/alliinase system

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ABSTRACT

In this work, we studied the catalytic kinetics of the alliin/alliinase system using a fiber-optic in situ monitoring system (fiber-optic drug dissolution test system, FODT) to explore the application of a fiber-optic sensor analysis technique in the process of monitoring catalytic kinetics. According to Lambert–Beer's law and with the assistance of the computational relationship of alliin, allicin and pyruvate, we established two mathematical models, including obtaining the absorptivity of each substance in the reaction and looking at the products as a whole. According to the UV spectra of alliin, alliinase, sodium pyruvate, an allicin solution and the analyte corresponding to the allicin peak extracted from an HPLC/PDA chromatogram, an optical probe with a 5-mm gap was chosen for the measurement, and 230 nm was chosen as the detection wavelength during the catalysis reaction. Then, we determined the value of every parameter in the mathematical model at 230 nm and recorded the information into the workstation. A certain concentration of alliinase was well-mixed with a series of alliin solutions in the volume ratio 1:1, and the concentration variation of alliin was monitored in real time. The maximum reaction rates for various concentrations of the substrate were obtained using Origin 7.5 software. To this end, a double-reciprocal plot was used to calculate the parameters of the catalytic kinetics of the alliin/alliinase system, including the Michaelis constant (K_m) and the maximum velocity (V_{max}), which was compared to HPLC results.

1. Introduction

Garlic (*Allium sativum* L.) is used world-wide as medicine-food treasure for a long history. Modern medicine has proved that garlic and its extract have unique and magical effect on infection, cardiovascular disease and tumor [1,2]. The active principle of garlic is assumed to be related to allicin (allyl-2-propenethiosulfinate), which is a product of the enzymatic reaction of alliinase (EC 4.4.1.4) with odorless precursor alliin (*S*-(+)-allyl-L-cysteine sulfoxide), as shown in following scheme (Scheme 1) [3]. Under natural conditions in a garlic-plant cell, the enzyme alliinase resides in micro-compartments separated by thin membranes and is thus physically separated from its substrate alliin. Crushing or injuring a garlic bulb breaks down the compartmentalization and brings the enzyme and its substrate into contact, leading to allicin production.

We have explored an extract of the active precursor alliin and alliinase from garlic, of which alliin is natural and its chemical name is (+)*S*-allyl-L-cysteine sulfoxide [4,5]. The catalytic kinetics of the alliin/alliinase system is challenging, and previous studies are summarized in Table 1, from which we can see that the K_m value of alliinase varies with the source of the alliinase and the substrate and ranges from 0.483 to 6.02 mM. The routine determination for the Michaelis constant was sampling analysis. Here, we described a method of in situ detection of the change of alliin by a fiber-optic in situ monitoring system (fiber-optic drug dissolution test system, FODT, Fig. 1), which is composed of a light source, a bifurcated optic fiber, a CCD detector and a relative software system [19]. FODT is a specified instrument for drug dissolution in situ monitoring system. A dissolution test is a dynamic-state process. Chemical reaction and catalytic reaction are also dynamic-state reaction processes. Based on the spectral analysis during the catalysis process and with the assistance of the computational relationship of alliin, allicin and pyruvate, the change in the alliin was detected in real time with

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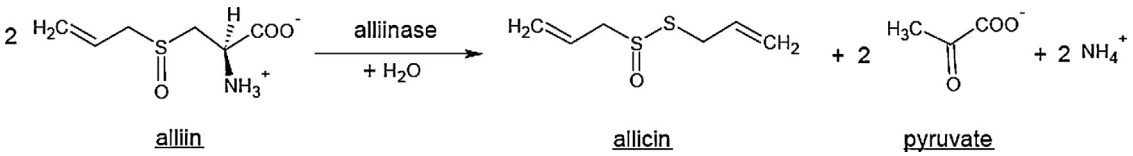

Scheme 1. The alliinase catalyst system.

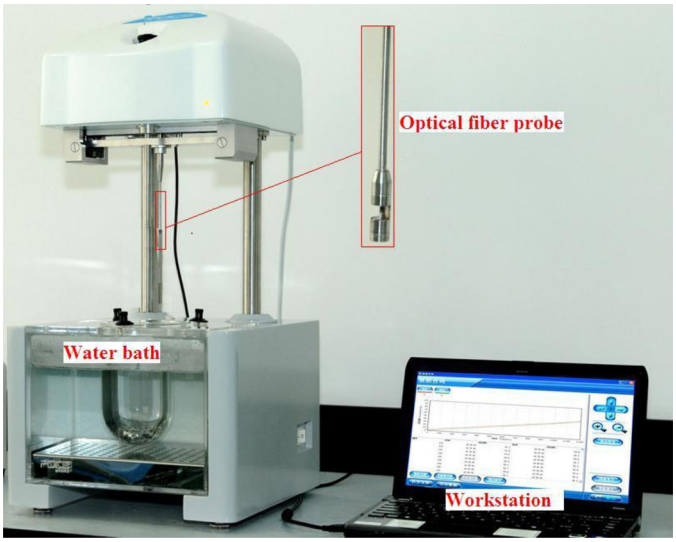

Fig. 1. The fiber-optic in situ monitoring system.

the FODT instrument. Then, the parameters of the catalytic kinetics of the alliin/alliinase system were calculated.

2. Experimental

2.1. Chemicals and materials

Alliin (purity of 99%) and alliinase raw materials were provided by Xinjiang Ailexin Pharma. Co., Ltd.; the activity of the alliinase was 1000 U/g. First, 10.20 mg of alliin were precisely weighed in a 10-mL volumetric flask, dissolved with distilled water and diluted to a series of solutions with concentrations of 183.6, 102, 81.6, 51, 40.8, 25.5, 20.4 and 10.2 μg/mL. Then, 10.23 mg of alliinase were precisely weighed in a 10-mL volumetric flask, dissolved with distilled water and diluted to concentrations of 26.20, 20.26 and 10.13 μg/mL. Next, 10.10 mg of sodium pyruvate were precisely weighed in a 10-mL volumetric flask, dissolved with distilled water and diluted to a series of solutions with concentrations of 96.96, 48.48 and 12.12 μg/mL. Five milliliter of the alliin solution with a concentration of 2 mg/mL were mixed with an equal volume of alliinase with a concentration of 2 mg/mL. This mixture was allowed to stand at room temperature for 30 min. Then, it was centrifuged in an ultrafiltration centrifuge tube in a refrigerated centrifuge at 12,000 × *g* for 30 min to obtain an allicin solution separated from the proteins. The allicin solution was diluted to a series with distilled water. The concentrations of allicin were determined using HPLC.

HPLC-grade acetonitrile and methanol were obtained from Sigma-Aldrich (Madrid, Spain). HPLC-grade tetrahydrofuran was obtained from Tianjin Zhiyuan Chemical Reagent Co., Ltd. (Tianjin, China). Guarantee-grade sodium dihydrogen phosphate was purchased from Tianjin Fuchen Chemical Reagents Factory (Tianjin, China). Trifluoroacetic acid, butylparaben, formic acid, sodium hydroxide and 1,4-dioxane were all analytical grade. Chemically pure *o*-phthalaldehyde was purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). All analytical-grade and chemically pure reagents were used without further purification. Ultrapure water was obtained from a Millipore water purification system (≥18 MΩ, Milli-*Q*, Millipore).

2.2. Apparatus

A fiber-optic drug dissolution test system (FODT-101) was developed by Chen et al. of Xinjiang Medical University and Shanghai FOCS Analytical Instruments Co. Ltd. A PS-1000 pair stirrer was obtained from EYELA (Tokyo, Japan). Ultrafiltration centrifuge tubes with a filter membrane with a 5000 molecular weight cut-off were purchased from Sartorius (Goettingen, Germany). A MULTIFUGEX-3R refrigerated centrifuge was obtained from Thermo Fisher (Waltham, MA, USA). The HPLC/PDA analyses

Table 1
Parameters of the catalytic kinetics of the alliin/alliinase system in previous studies.

Reference	Source of alliinase	Substrate	Measurement index/method	K_m (mM)	V_{max}
[6]	Garlic	Synthetic *S*-allylcysteine sulfoxide (+isomer)	–	1.1	–
[7]	Garlic	Alliin(+)-isomer	–	1.8	–
[8]	Garlic	(±)-*S*-allyl-L-cysteine sulphoxide	–	6	123 units/mg of protein
[9]	Chinese garlic	Racemic alliin	Pyruvate/UV	2.9	27.8 milliunits/min
[10]	Garlic powder	L-(+)-Alliin	Pyruvate/HPLC	1.56	252 μmol/(mg min)
		L-(−)-Alliin	Pyruvate/HPLC	2.80	54 μmol/(mg min)
	Fresh garlic	L-(+)-Alliin	Pyruvate/HPLC	1.11	332 μmol/(mg min)
		L-(−)-Alliin	Pyruvate/HPLC	2.28	90 μmol/(mg min)
[11]	Garlic	Synthesis *S*-allyl-L-cysteine sulfoxide	Pyruvate/–	3.3	–
[12]	Garlic	Natural diastereoisomer of alliin	–	0.5	–
[13]	Garlic	Synthetic (±)-diastereomer *S*-allyl-L-cysteine sulfoxide	Pyruvate/UV	2.2	20 nmol/min
[14]	Garlic	Synthesis *S*-allyl-L-cysteine sulfoxide	Pyruvate/UV	6.02	120.07 U/mg
[15]	Garlic	Synthesis *S*-allyl-L-cysteine sulfoxide	Pyruvate/UV	5.91	1.55 μmol/min
[16]	Garlic	Synthesis *S*-allyl-L-cysteine sulfoxide	Pyruvate/UV	4.17	156.25 units/mg. of protein
[17]	Garlic	Natural extract	Pyruvate/UV	0.483	0.492 IU/mg
[18]	Garlic	Racemic alliin	Pyruvate/UV	0.693	0.353 mmol/min