Application Note

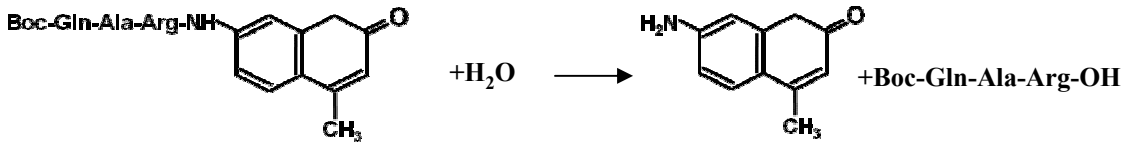
FP-0011

Activity measurement of trypsin using a fluorescence peptide substrate

Introduction

Hydrolysis reaction is caused by making protease act on METHYLCOUMARIN-AMIDE (MCA) of peptide substrate, and 7-AMIDO-4-METHYLCOUMARIN (AMC) isolated.

This isolated AMC, fluorescence becomes the maximum by wavelength 440 nm. Protease activity can be measured using fluorescence spectrophotometer. We introduce the example which performed activity measurement of trypsin using a fluorescence peptide MCA substrate.



Boc-Gln-Ala-Arg-MCA　　Isolation AMC

Fig. 1 The hydrolysis reaction of MCA by protease

Keywords: Kinetics, Enzyme activity, Lineweaver-Burk, Michaelis-Menten

Measurement system

FP-8300	Spectrofluorometer
STR-812	Water thermostatted cell holder with stirrer
CSP-829	Sample compartment lid with syringe port
MCB-100	Mini Circulation Bath*1)
VWKN-772	Kinetics Analysis Program

*1) The temperature of a circulation bath is set as 37 degrees by all the measurement.

Samples

Intensity standardization sample: 50 µmol/L AMC solution

Enzyme solution: 10 nmol/L Trypsin bovine pancreas typeVIII, 50 mmol/L Tris-HCl, 0.15 mol/L NaCl, 1.0 mmol/L $CaCl_2$, 0.1 mg/mL BSA

Substrate solution: Boc-Gln-Ala-Arg-MCA solution

(The concentration after mixture is adjusted to 0.5, 1, 2.5, 5, 10, 20, 40 µmol/L)

Concentration for adjustment. [µmol/L]	240	120	60	30	15	6	3
The last concentration. [µmol/L]	40	20	10	5	2.5	1	0.5

Measurements

(1) Fluorescence-spectrum measurement of AMC

Excitation and the Fluorescence spectrum of 50 µmol/L AMC were measured (Fig. 2). It turns out that the fluorescence maximum wavelength from this result is 440 nm.

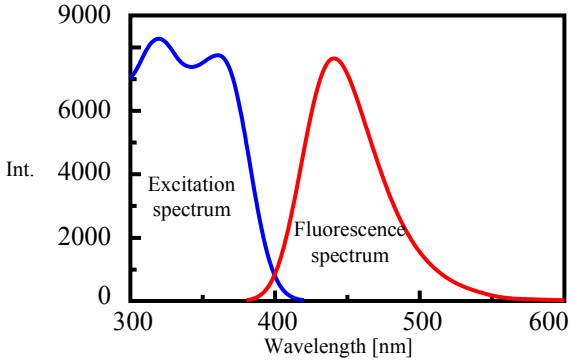


Fig. 2 Fluorescence spectrum of AMC

[Measurement parameters]
Ex wavelength: 360 nm
Em wavelength:440 nm
Ex bandwidth: 5 nm
Em bandwidth: 10 nm
Response: 0.5 sec
Sensitivity: 200 V
Data interval: 1 nm
Scan speed 500 nm/min

JASCO INTERNATIONAL CO., LTD

4-21, Sennin-cho 2-chome, Hachioji, Tokyo 193-0835, Japan
Tel: +81-42-666-1322, Fax: +81-42-665-6512

(2) The vertical axis is changed into concentration from fluorescence intensity

Intensity standardization was performed in order to change the vertical axis into the numerical value equivalent to concentration.

50 µmol/L AMC solution of 0.5 mL is dropped at enzyme solution 2.5 mL. Fluorescence intensity of last concentration of 8.333 µmol/L AMC solution was set to 8333 µmol/L AMC solution.

(3) Enzyme activity measurement

Substrate solution 0.5 mL of each concentration was dropped at enzyme solution 2.5 mL, and time course measurement of the fluorescence intensity of the isolation AMC was performed to it. A result is shown in Fig. 3.

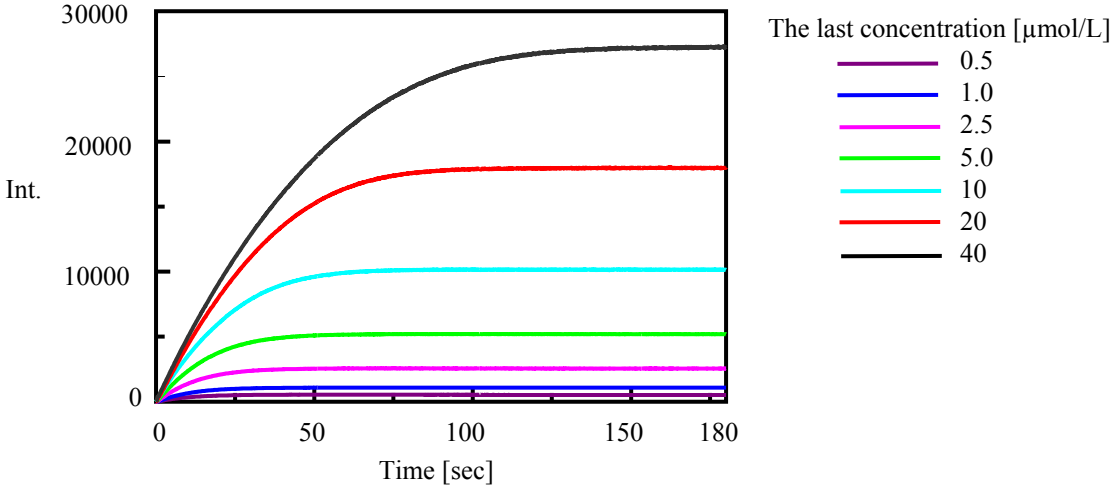


Fig. 3 The pursuit result of an enzyme reaction process

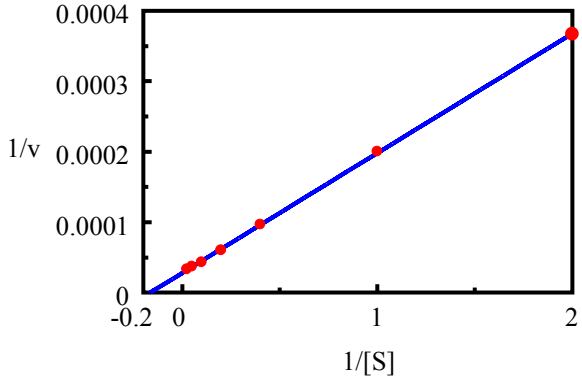
[Measurement parameters]

Ex wavelength:	360 nm	Em wavelength:	440 nm
Ex bandwidth:	5 nm	Ex bandwidth:	10 nm
Data interval:	0.1 sec	Response:	0.1 sec
Scan speed:	200 V		

Analysis

[Kinetics Analysis] In quest of each initial velocity, the Lineweaver-Burk plot was performed using the program from inclination of the time variation data of each substrate concentration (Fig. 4).

Km=5.99 and Vmax=35270 nmol/L-min^{-1} were obtained from this result.



[Calculation result]

Calculation method:	Lineweaver-Burk Plot
Vmax:	35270
Km:	5.99

$1/v = 0.000170 \times 1/[S] + 0.0000284$

Fig. 4 Lineweaver-Burk plot

JASCO INTERNATIONAL CO., LTD

4-21, Sennin-cho 2-chome, Hachioji, Tokyo 193-0835, Japan
Tel: +81-42-666-1322, Fax: +81-42-665-6512