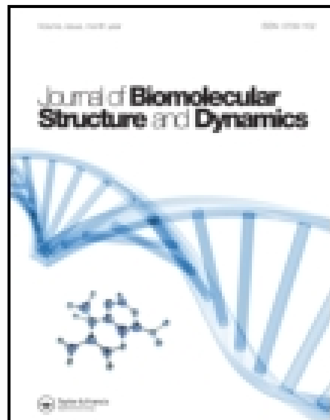
This article was downloaded by: [Zhejiang University]
On: 19 March 2015, At: 04:13
Publisher: Taylor & Francis
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Biomolecular Structure and Dynamics
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tbsd20

Inactivation Kinetics of β-N-Acetyl-D-glucosaminidase from Green Crab (Scylla serrata) in Dioxane Solution

Jin-Jin Xie [a] [b] , Chao-Qi Chen [c] , Ya-Wen Yan [a] , Ji-Ping Zhang [a] , Jian-Cheng Lin [a] , Qin Wang [a] , Han-Tao Zhou [a] & Qing-Xi Chen [a]

[a] Key Lab of Ministry of Education for Cell Biology and Tumor Cell Engineering , School of Life Sciences Xiamen University , Xiamen , 361005 , China

[b] Biology Department , Quanzhou Normal University , Quanzhou , 362000 , China

[c] College of Environmental Sciences Peking University , Peking , 100871 , China

Published online: 15 May 2012.

To cite this article: Jin-Jin Xie , Chao-Qi Chen , Ya-Wen Yan , Ji-Ping Zhang , Jian-Cheng Lin , Qin Wang , Han-Tao Zhou & Qing-Xi Chen (2009) Inactivation Kinetics of β-N-Acetyl-D-glucosaminidase from Green Crab (Scylla serrata) in Dioxane Solution, Journal of Biomolecular Structure and Dynamics, 26:4, 509-515, DOI: 10.1080/07391102.2009.10507266

To link to this article: http://dx.doi.org/10.1080/07391102.2009.10507266

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at http://www.tandfonline.com/page/terms-and-conditions

Journal of Biomolecular Structure & Dynamics, ISSN 0739-1102
Volume 26, Issue Number 4, (2009)
©Adenine Press (2009)

Downloaded by [Zhejiang University] at 04:13 19 March 2015

Inactivation Kinetics of β-N-Acetyl-D-glucosaminidase from Green Crab (*Scylla serrata*) in Dioxane Solution

http://www.jbsdonline.com

Jin-Jin Xie[1,2]
Chao-Qi Chen[3]
Ya-Wen Yan[1]
Ji-Ping Zhang[1]
Jian-Cheng Lin[1]
Qin Wang[1]
Han-Tao Zhou[1,*]
Qing-Xi Chen[1,*]

[1]Key Lab of Ministry of Education for Cell Biology and Tumor Cell Engineering
School of Life Sciences
Xiamen University
Xiamen 361005, China
[2]Biology Department
Quanzhou Normal University
Quanzhou 362000, China
[3]College of Environmental Sciences
Peking University
Peking 100871, China

Abstract

β-N-Acetyl-D-glucosaminidase (NAGase, EC.3.2.1.52), which catalyzes the cleavage of N-acetylglucosamine polymers, is a composition of chitinase and cooperates with endo-chitinase and exo-chitinase to disintegrate chitin into N-acetylglucosamine (NAG). In this investigation, A NAGase from green crab (*Scylla serrata*) was purified and the effects of dioxane on the enzyme activity for the hydrolysis of *p*-Nitrophenyl-N-acetyl-β-D- glucosaminide (pNP-NAG) were studied. The results show that appropriate concentrations of dioxane can lead to reversible inactivation of the enzyme and the inactivation is classified as mixed type. The value of IC_{50}, the dioxane (inactivator) concentration leading to 50% activity lost, is estimated to be 0.68%. The kinetics of inactivation of NAGase in the appropriate concentrations of dioxane solution has been studied using the kinetic method of the substrate reaction. The rate constants of inactivation have been determined. The results showed that k_{+0} is much larger than k'_{+0}, indicating the free enzyme molecule is more fragile than the enzyme-substrate complex in the dioxane solution. It is suggested that the presence of the substrate offers marked protection of this enzyme against inactivation by dioxane.

Key words: β-N-Acetyl-D-glucosaminidase; *Scylla serrata*; Dioxane; Inactivation; Kinetics.

Introduction

Chitin, a polymer of N-acetylglucosamine (NAG), is the abundant carbon resource in the nature. Digestion of chitin to NAG is carried out by chitinase and β-N-Acetyl-D-glucosaminidase (NAGase, EC 3.2.1.52). Chitinase catalyzes the hydrolysis of chitin into the dimer and trimer oligomers of NAG, which will be disassembled by NAGase into the monomer. In marine species, chitinases are particularly associated with the molting processes of arthropods and crustaceans (1, 2). NAGase has been studied extensively because of its important roles in many fields, such as defense systems against parasites, molting cycle and digestion of chitinous foods, and so on (3, 4). NAGases in Antarctic krill (1), prawn (*Penaeus vannamei*) (5, 6), and fiddler crab (*Uca pugilator*) (2) have also been characterized in terms of purification, characteristics, their concentrations in different growth stage and distribution in different organs, respectively. Zou (2) found that NAGase activity in the epidermis and hepatopancreas from fiddler crab was well correlated with the hemolymph titer of ecdysteroids during the molting cycle, suggesting that NAGase activity in both tissues is regulated at least in part by the steroid molting hormones. Green crab (*Scylla serrata*) is a most important economic marine crustacean animal. We discovered that green crab has very high NAGase activity in its viscera. In our previous studies, we reported the purification and some enzymatic characterization of NAGase from

* Q.-X. Chen, chenqx@xmu.edu.cn
H.-T. Zhou, htzhou@xmu.edu.cn

Abbreviations: NAGase, β-N-acetyl-D-glucosaminidase; *p*NP-NAG, *p*-nitrophenyl-N-acetyl-β-D-glucosaminide; NAG, N-acetylglucosamine; pNP, *p*-nitrophenol; PAGE, polyacrylamide gel electrophoresis; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; IC_{50}, the inhibitor concentration leading to 50% of enzyme activity lost; k_{+0} and k'_{+0} are the inactivated rate constants of free enzyme and enzyme-substrate complex, respectively.

Downloaded by [Zhejiang University] at 04:13 19 March 2015

the viscera of green crab (7). And the inhibitory kinetics of mercuric ion (8), formaldehyde (9), phenol (10), and hydrogen peroxide (11) on this enzyme has been well-studied. The nature environment of most proteins is the complex system contained in all varieties of organic compounds such as sugar, fat, organic acid, alcoholate, and so on. Meanwhile, because of pollution of the breeding aquatic environment, such as a shift of acidity and alkalinity and heavy metal ions or organic solvents, the enzyme activity and its conformation may be affected, and the growth and survival of the animal is threatened (10). So it is very important to research the influence of organic solvents on the enzyme activity and the enzyme performance change in organic solvents. Up to the present, the effect of organic solvents on NAGase has hardly been reported. In our continuous investigation, dioxane was found to have strong effect on the enzyme activity and its conformation. The inactivation of the enzyme in dioxane solution was showed to be reversible. The aim of this current paper is, therefore, to carry out a kinetics study on the inactivation of NAGase in the dioxane solution. It has significant instruction to inspect the pollution of the breeding aquatic environment by utilizing high delicacy of enzyme to organic solvents.

Materials and Methods

Materials

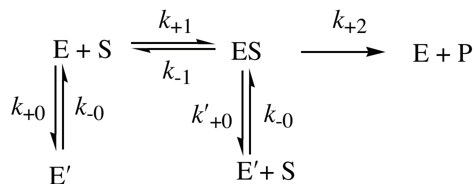
p-Nitrophenyl-N-acetyl-β-D-glucosaminide (*p*NP-NAG) was purchased from the Biochemistry Lab of Shanghai Medicine Industry Academy (China). Sephadex G-100 was supplied from Pharmacia products. DEAE-cellulose (DE-32) was from Whatman. Standard marker proteins were from Amersham. Dioxane was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Other reagents were local products of analytical grade. The water used was re-distillated and ion-free.

Enzyme Assay

NAGase (EC 3.2.1.52) was prepared from green crab (*Scylla serrata*) as described previously (7). The crude preparation was further chromatographed by gel filtration through Sephadex G-100 followed by DEAE-cellulose (DE-32). The final preparation was homogeneous on PAGE and SDS-PAGE. The specific activity of the purified enzyme was 7990 U/mg. Enzyme concentration was determined as described by Lowry (12). The enzyme activity assay was performed with *p*NP-NAG as substrate by the methods described by Zhang *et al*. (7) with slight modifications. In this method, 10 μl of NAGase was added to 3.0 ml of an assay system containing 0.5 mM of *p*NP-NAG and 0.05 M NaAc-HAc buffer (pH 5.8). The enzyme activity was calculated from the linear increase in optical density at 405 nm with the *p*NP molar absorption coefficient of $1.77 \times 10^3\ M^{-1} \cdot cm^{-1}$ at pH 5.8 (7). Absorption was recorded using a Beckman UV-650 spectrophotometer. One unit (U) of enzymatic activity was defined as the amount of enzyme required to produce 1 μmol of *p*NP per minute per liter under above conditions.

Inactivation Rate Constants of the Enzyme in Dioxane Solutions

The substrate reaction method previously described (13, 14) was used to study the inactivation kinetics of the enzyme in dioxane solutions. In this method, 10 μl of the purified enzyme (0.15 μg/ml) was added to 3.0 ml of an assay system containing different concentrations of substrate in 0.05 M NaAc-HAc buffer (pH 5.8) with different concentration of dioxane. The substrate reaction progress curve was analyzed to obtain the rate constants as detailed below. The reaction was carried out at a constant temperature of 37 ºC. The time course of the hydrolysis of the substrate in different concentrations of dioxane solution showed that the rate decreased with increasing time until a straight line was approached. The results showed that the inactivation was a reversible reaction with fractional residue activity. This can be written as the following scheme (14):



Where S, P, E and E′ denote substrate, product, native enzyme, and inactivated enzyme, respectively; ES are enzyme-substrate compounds. k_{+0} and k'_{+0} are the inactivated rate constants of free enzyme and enzyme-substrate complex, respectively. $[E_0]$ denotes the initial enzyme concentration, and [S] denotes the concentrations of substrate. As is usually the case, $[S]>>[E_0]$ and we can get the following equation from the scheme:

$$\frac{-d[E_T]}{dt} = \frac{d[E']}{dt} = k_{+0}[E] + k'_{+0}[ES] - k_{-0}[E'] \quad [1]$$

and

$$[E_0] = [E] + [ES] + [E'] = [E_T] + [E'] \quad [2]$$
$$[E_T] = [E] + [ES]$$

Where, $[E_T]$ denotes the concentrations of the active enzyme.

$$[E] = \frac{K_m}{K_m + [S]}[E_T] \quad [3]$$

$$[ES] = \frac{[S]}{K_m + [S]}[E_T] \quad [4]$$

Combining Eqs. [2], [3], and [4], Eq. [1] could be changed into the below equation.

$$\frac{-d[E_T]}{dt} = \left(\frac{k_{+0}K_m + k_{+0}'[S]}{K_m + [S]} + k_{-0}\right)[E_T] - k_{-0}[E_0] = A \cdot [ET] - k_{-0}[E_0] \quad [5]$$

and

$$A = \frac{k_{+0} \cdot K_m + k'_{+0}[S]}{K_m + [S]} + k_{-0} \quad [6]$$

From Eq.[5] we can obtain:

$$\frac{[E_T]}{[E0]} = \frac{A - k_{-0}}{\mathrm{A}} \cdot e^{-A \cdot t} + \frac{k_{-0}}{\mathrm{A}}$$
$$v_t = \frac{d[P]}{dt} = \frac{[E_T]}{[E_0]} \cdot v \quad [7]$$

The product formation can be written as:

$$[P]_{\mathrm{t}} = \frac{k_{-0} \cdot v}{A} \cdot t + \frac{v}{A^2}(A - k_{-0})(1 - e^{-A \cdot t}) \quad [8]$$

Where $[P]_t$ is the concentration of the product formed at time t, which is the reaction time; A is the apparent rate constants for the forward reaction of inactivation, respectively; [S] is the concentrations of the substrate; v is the initial rate of reaction in the absence of dioxane and $v = \frac{V_m \cdot [S]}{K_m + [S]}$. When t is sufficiently large, the curves become straight lines and $[P]_t$ is written as $[P]_{calc}$:

$$[P]_{\mathrm{calc}} = \frac{k_{-0} \cdot v}{A} \cdot t + \frac{v}{A^2}(A - k_{-0}) \quad [9]$$

Downloaded by [Zhejiang University] at 04:13 19 March 2015

Downloaded by [Zhejiang University] at 04:13 19 March 2015

Combining Eqs. [8] and [9] yields

$$[P]_t = \frac{k_{-0} \cdot v}{A} \cdot t + \frac{v}{A^2}(A - k_{-0})(1 - e^{-A \cdot t}) \qquad [10]$$

$$\ln([P]_{calc} - [P]_t) = -A \cdot t + \ln \frac{v}{A^2}(A - k_{-0}) \qquad [11]$$

Where $[P]_{calc}$ is the product concentration to be expected from the straight-line portions of the curves as calculated from Eq. [9] and $[P]_t$ is the product concentration actually observed at time t. Plots of $\ln([P]_{calc}-[P]_t)$ versus t give a series of straight lines at different concentrations of denaturant with slopes of -A. The apparent forward rate constant A can be obtained from the graphs. From Eq. [9], a plot of $[P]_{calc}$ against time, t, gives a straight line with a slope of $\frac{k_{-0} \cdot v}{A}$. From the slope of the straight line, k_{-0} can be obtained.

Combining Eq. [6] and the Michaelis-Menten equation gives

$$\frac{A}{v} = \frac{K_m}{V_m}(k_{+0} + k_{-0}) + \frac{1}{[S]} + \frac{k'_{+0} + k_{-0}}{V_m} \qquad [12]$$

A plot of A/v *versus* 1/[S] gives a straight line with $\frac{K_m}{V_m}(k_{+0} + k_{-0})$ and $\frac{k'_{+0} + k_{-0}}{V_m}$ as the slope and intercept, respectively. As K_m and V_m are known quantities from measurements of the substrate reaction in the absence of denaturant at different substrate concentrations and k_{-0} can be obtained from a suitable plot as above, the rate constants k_{+0} and k'_{+0} can be obtained from the slope and intercept of the straight line, respectively.

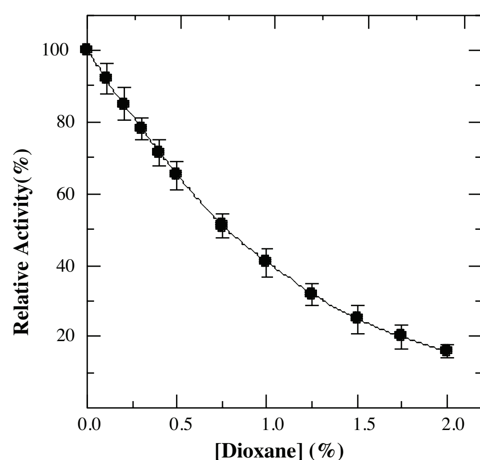


Figure 1: Effect of dioxane on the activity of NAGase for the hydrolysis of *p*NP-NAG. Assay conditions were: 2 ml system containing 0.15 M of NaAc-HAc buffer (pH 5.8) and 0.50 mM of pNP-NAG at 37 ºC. The enzyme final concentration was 0.15 μg/ml.

Results

Effect of Dioxane on the Enzyme Activity

The effect of dioxane on the hydrolysis of pNP-NAG by green crab NAGase was first studied. The relationship between the residual enzyme activity and the concentrations of dioxane was shown in Figure 1. The effect of dioxane on the enzyme was concentration dependent. With increasing the concentrations of dioxane, the NAGase activity markedly decreased. The IC_{50} value, the inhibitor concentration leading to 50% of enzyme activity lost, was estimated to be 0.68%. Figure 2 showed the relationship of enzyme activity with its concentration in the presence of different concentrations of dioxane. The plots of the remaining enzyme activity versus the concentrations of enzyme in the presence of different concentrations of dioxane gave a family of straight lines, which all passed through the origin. Increasing the dioxane concentration resulted in the descending of the slope of the line, indicat-

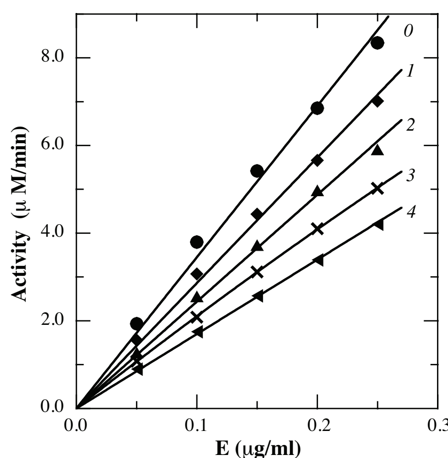


Figure 2: Effects of NAGase concentration on its activity for the hydrolysis of *p*NP-NAG at different concentrations of dioxane. The concentrations of dioxane for curves 0-4 were 0, 0.2, 0.4, 0.6, and 0.8 %, respectively. Assay conditions were same as Figure 1 except that the enzyme concentration was changed.

ing that the inactivation of the enzyme in dioxane solution was reversible reaction course. The presence of dioxane did not bring down the amount of the efficient enzyme, but just resulted in the inhibition and the descending of the activity of the enzyme. Dioxane is reversible inactivator of NAGase for hydrolysis of pNP-NAG.

Kinetics of the Substrate Reaction of the Enzyme in Different Concentrations of Dioxane Solutions

The kinetic behavior of NAGase in catalyzing the hydrolysis of pNP-NAG has been well studied (7, 8). Under the condition employed in the present investigation, the hydrolysis of pNP-NAG by NAGase follows Michaelis-Menten kinetics. The kinetic parameters were determined, and the results showed that K_m was 0.44 ± 0.02 mM and V_m was equal to 14.97 ± 0.50 μM/min.

The temporal variation of the product concentration during the substrate hydrolysis in the presence of different dioxane concentrations was shown in Figure 3a. At each concentration of dioxane, the rate decreases with increasing time until a straight line is approached, the slope of which decreased with increasing dioxane concentration. The results suggest that denatured NAGase still had partial residue activity (curves 1-5). According to Eq. [11], plots of $\ln([P]_{calc}-[P]_t)$ versus t which give a series of straight lines were shown in Figure 3b. From the slopes of the straight line, the apparent forward rate constant of inactivation, A, can be obtained.

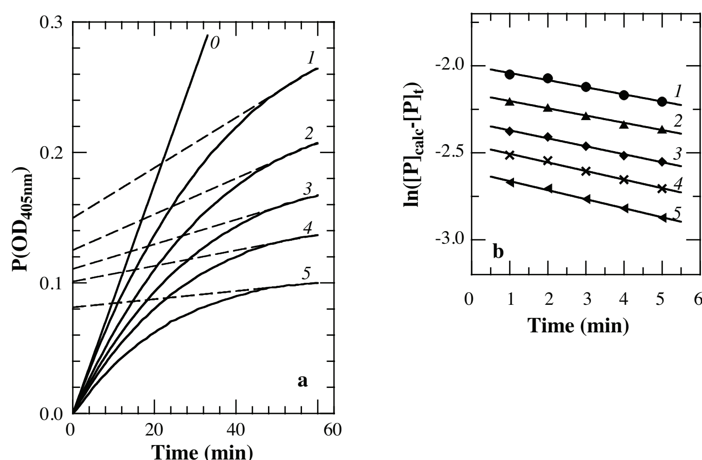


Figure 3: Course of inactivation of NAGase for the hydrolysis of pNP-NAG in different concentrations of dioxane solution. The assay conditions were the same as Figure 1 with exception of 0.25 mM pNP-NAG. (**a**) Substrate reaction course. The final dioxane concentrations for curves 0-5 were 0, 0.2, 0.4, 0.6, 0.8, and 1.0%, respectively. The broken straight line slowed the plot of $[P]_{calc}$ against time t with a slope of $\frac{v \cdot k_{-0}}{A}$. (**b**) Semi-logarithmic plots of $\ln([P]_{calc}-[P]_t)$ against time. Data were taken from curves 1-5 in (**a**).

Downloaded by [Zhejiang University] at 04:13 19 March 2015

Measurement of Inactivation Rate Constant of NAGase in Dioxane Solution

The kinetic course of the pNP-NAG hydrolysis reaction by the enzyme in different concentrations of dioxane solution was first studied. Figure 4 showed the enzyme kinetics in 0.2% dioxane with different substrate concentrations. In the presence of 0.2% of dioxane, when the time is sufficiently large, a straight line is approached at each concentration of substrate. Both the initial rate and the slope of the asymptote increase with increasing substrate concentration (Fig. 4a). From Eq. [11], plots of $\ln([P]_{calc}-[P]_t)$ versus t give a series of straight lines at different concentrations of substrate, whose slopes are equal to the apparent forward rate constant A (Fig. 4b). From Eq. [9], a plot of $[P]_{calc}$ against time t gives a straight line with a slope of $\frac{v \cdot k_{-0}}{A}$. Because the value of v was obtained by testing at different concentrations of substrate, the value of k_{-0} can be obtained.

Since the values of initial rate (v), Michaelis-Menten constant (K_m) and maximal rate constant (V_m) are quantities known from measurement of the substrate reaction in the absence of dioxane at different substrate concentrations, the inactivation rate constants k_{+0} and k'_{+0} were obtained from the slope and the intercept of the straight line in Figure 4c, a plot of A/v versus 1/[S] according to Eq. [12]. The values

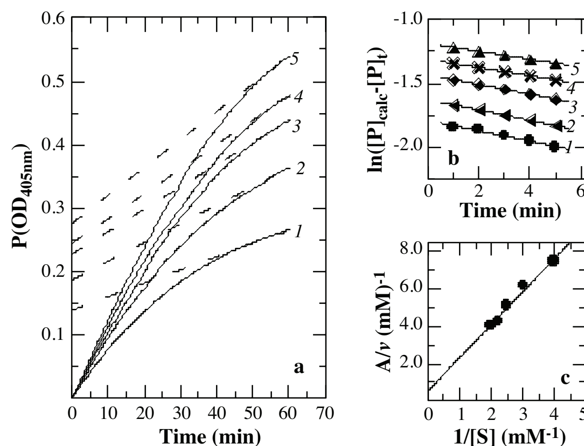


Figure 4: Determination of the inactivation rate constants for crab NAGase in 0.2% of dioxane solution. (**a**) Substrate reaction courses of the enzyme in the presence of 0.2% dioxane. Curves 1-5 are progress curves with 0.25, 0.33, 0.40, 0.45, 0.50 mM of substrate, respectively. (**b**) Semilogrithmic plot of $\ln([P]_{calc}-[P]_t)$ against time. Data were taken from curves 1-5 in (**a**). (**c**) Plot of A/v versus 1/[S], the A values were obtained from the slopes of the straight lines in (**b**).

obtained are listed in the Table I. Similarly, the inactivation rate constants of the enzyme at other dioxane concentrations were also obtained (Table I).

Table I
Microscopic rate constants of the inactivation of *Scylla serrata* NAGase in dioxane solutions.

[dioxane] (%)	rate constants ($\times 10^{-3}$ s^{-1}) k_{+0}	k_{-0}	k'_{+0}	residual activity (%)	fluorescence intensity (%)
0	0	0	0	100.00	100.00
0.2	49.41 ± 1.20	9.26 ± 0.50	1.40 ± 0.10	83.00 ± 2.00	94.14 ± 2.50
0.4	53.02 ± 1.20	6.67 ± 0.40	5.41 ± 0.15	67.50 ± 1.50	74.98 ± 2.50
0.6	59.18 ± 1.00	5.35 ± 0.35	8.22 ± 0.24	54.20 ± 1.40	58.11 ± 1.80
0.8	66.17 ± 0.80	3.11 ± 0.30	12.25 ± 0.54	44.20 ± 0.80	48.91 ± 1.50
1.0	69.38 ± 0.70	2.44 ± 0.25	14.95 ± 0.55	36.00 ± 1.00	44.96 ± 0.80

Downloaded by [Zhejiang University] at 04:13 19 March 2015

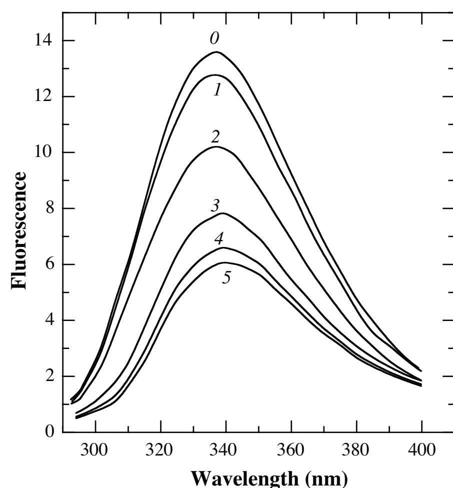


Figure 5: Fluorescence emission spectra of NAGase inactivated in the dioxane solutions. Enzyme (10 µl of 1.106 mg/ml) was added into 1ml of 0.1 M NAc-HAc buffer pH 5.8 containing different concentrations of dioxane before determination of the fluorescence spectra. The excitation wavelength was 278.8 nm. The enzyme concentration was 11.06 µg/ml. The dioxane concentration in the inactivation mixture for curves 1-5 were 0, 0.2, 0.4, 0.6, 0.8, and 1.0%, respectively.

Fluorescence Emission Spectra of the Enzyme After Denaturation in Dioxane Solutions of Different Concentrations

The fluorescence emission spectra of the enzyme in different concentrations of dioxane are shown in Figure 5. The emission peak of the native enzyme is at 339.4 nm; it may contain contributions by both Trp residues and Tyr residues (14). Increasing the concentrations of dioxane caused the fluorescence emission intensity to be markedly decreased and the emission peak at 339.4 nm a little red-shifted. When the dioxane concentration reached to 1.0%, the fluorescence intensity decreased from 13.470 to 6.057, decreased by 45.04%, and the red shift of the emission maximum was only 1.5 nm. The results indicated that conformational changes of the enzyme molecule occurred in dioxane solution, which led to the inactivation of the enzyme.

Dicussion

Enzymes and proteins in organic solvents were received expanding attention in the past decade, and some novel properties have been reported while enzymes work in organic solvents (16, 17), which benefit both biotechnology and pharmaceutical industry. In this investigation, we found that NAGase from green crab (*Scylla serrata*) could be affected in dioxane solutions and the inactivation of the enzyme was showed to be reversible. We did not only use the substrate reaction kinetic method to analyze the inhibitory kinetics of NAGase in dioxane solution, but also elucidate the relationship between the changes of conformation and function of the enzyme at different concentrations of dioxane. The results listed in Table I show that the values of k_{+0} and k'_{+0} increase apparently with increasing the dioxane concentration, but the value of k_{-0} decreases, illuminating the reversible intensity of enzyme is weakened with increasing the dioxane concentration. The value of k_{+0} is much larger than k'_{+0}, indicating the free enzyme molecule is more fragile than the enzyme-substrate complex in the dioxane solution (6, 14). When the concentration

of dioxane increased from 0.2% to 1.0%, there is about 41% increase for k_{+0} and 964% increase for k'_{+0}, indicating that, in the high concentration of dioxane solution, the substrate protection of this enzyme against inactivation is weakened. The residual activity and fluorescence intensity decrease with increasing the dioxane concentration. And, the fluorescence intensity decreases more slowly than the residual activity, indicating that the conformation changes of the whole enzyme were more slowly than that of the enzyme active center in the dioxane solution.

The change of the fluorescence intensity of green crab NAGase in dioxane solution is as same as that of prawn NAGase (6) but very different from that of mushroom tyrosinase in the DMSO (15), whose fluorescence intensity increased with increasing the concentration of DMSO. Maybe it is because the adding of dioxane causes the change of pH or dielectric constant, or the presence of dioxane could modify the nature and the number of non-covalent interaction, so the microenviroments of NAGase change and then its conformation is affected and leads to enzyme activity loss.

Acknowledgments

The present investigation was supported by Grants (40576066, and 30500102) of the Natural Science Foundation of China and by the Program for Innovative Research Team in Science and Technology in Fujian Province University.

Downloaded by [Zhejiang University] at 04:13 19 March 2015

References and Footnotes

1. G. Peters, R. Saborowski, F. Buchholz, and R. Mentlein. *Mar Biol 134*, 697-703 (1999).
2. E. Zou and M. Fingerman. *Mar Biol 133*, 97-101 (1999).
3. D. Koga, C. Shimazaki, and K. Yamamoto. *Agric Biol Chem 51*, 1679-1681 (1987)
4. P. J. Espie and J. C. Roff. *Physiol Zool 68*, 727-748 (1995).
5. X. L. Xie, Q. X. Chen, J. C. Lin, and Y. Wang. *Mar Biol 146*, 143-148 (2004).
6. X. L. Xie and Q. X. Chen. *Biochemistry (Moscow) 69*, 1675-1682 (2004).
7. J. P. Zhang, Q. X. Chen, Q. Wang, and J. J. Xie. *Biochem (Moscow) 71S*, 55-59 (2006).
8. J. J. Xie, Q. X. Chen, J. P. Zhang, Q. Wang, and X. M. Yang. *Int J Biol Macromol 39*, 159-164 (2006).
9. J. J. Xie, J. P. Zhang, Q. Wang, X. M. Yang, J. C. Lin, and Q. X. Chen. *J Xiamen Uni 45*, 98-101 (2006).
10. J. P. Zhang, Y. W. Yan, J. J. Xie, Q. Wang, and Q. X Chen. *Int J Biol Macromol 40*, 139-143 (2007).
11. J. J. Xie, J. C. Lin, J. P. Zhang, Y. W. Yan, Q. Wang, and Q. X. Chen. *J Oceanography in Taiwan Strait 25*, 459-464 (2006).
12. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall. *J Biol Chem 193*, 265-269 (1951).
13. Q. X. Chen, W. Zhang, W. Z. Zheng, H. Zhao, S. X. Yan, H. R. Wang, and H. M. Zhou. *J Protein Chem 15*, 345-350 (1996).
14. Q. X. Chen, Z. Zhang, H. Huang, F. K. Zhao, and G. J. Xu. *Int J Biochem & Cell Biol 35*, 1227-1233 (2003).
15. Q. X. Chen, X. D. Liu, and H. Huang. *Biochem (Moscow) 68*, 644-649 (2003).
16. Q. X. Chen, R. Q. Zhang, P. Z. Yang, Y. Li, S. L. Chen, S. Li, Y. Yang, and H. M. Zhou. *Int J Biol Macromol 26*, 103-107 (1999).

Date Received: July 9, 2008

Communicated by the Editor Ramaswamy H. Sarma