Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 121 (2014) 230–237

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

# Insights into the selective binding and toxic mechanism of microcystin to catalase

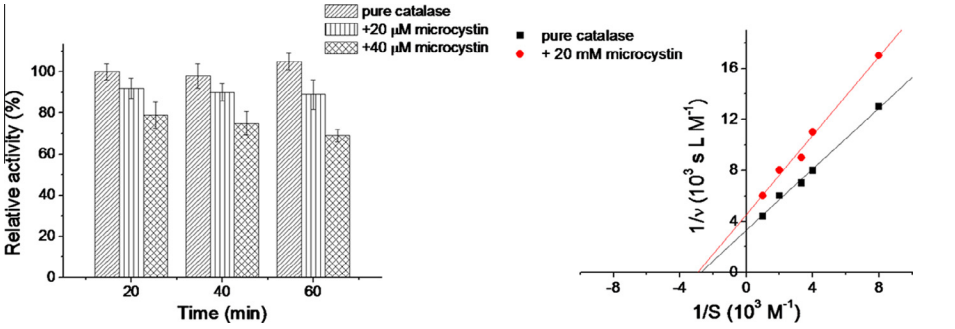
Yuandong Hu [a,b], Liangjun Da [a,*]

[a] Department of Environmental Science, East China Normal University, Shanghai 200062, China
[b] Department of Landscape, Northeast Forestry University, Harbin 150040, China

## HIGHLIGHTS

- Interaction of catalase and microcystin was studied.
- Binding of microcystin to catalase follows the electrostatic force.
- Conformational alterations of catalase were caused by microcystin binding.
- Activity of catalase was inhibited by noncompetitive mechanism.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 4 June 2013
Received in revised form 22 August 2013
Accepted 25 September 2013
Available online 19 October 2013

*Keywords:*
Cyanotoxin
Catalase
Fluorescence quenching
FT-IR
Enzymatic inhibition

## ABSTRACT

Microcystin is a sort of cyclic nonribosomal peptides produced by cyanobacteria. It is cyanotoxin, which can be very toxic for plants and animals including humans. The present study evaluated the interaction of microcystin and catalase, under physiological conditions by means of fluorescence, three-dimensional (3D) fluorescence, circular dichroism (CD), Fourier Transform infrared (FT-IR) spectroscopy, and enzymatic reactionkinetic techniques. The fluorescence data showed that microcystin could bind to catalase to form a complex. The binding process was a spontaneous molecular interaction procedure, in which electrostatic interactions played a major role. Energy transfer and fluorescence studies proved the existence of a static binding process. Additionally, as shown by the three-dimensional fluorescence, CD and FT-IR results, microcystin could lead to conformational and microenvironmental changes of the protein, which may affect the physiological functions of catalase. The work provides important insights into the toxicity mechanism of microcystin *in vivo*.



## Introduction

Cyanobacteria (formerly called "blue–green algae") have a worldwide distribution and can form extensive blooms in freshwater and estuarine habitat. Factors that contribute to bloom formation and toxin production include warm water [1], nutrient enrichment [2] and seasonal increases in light intensity [3]. Rising global temperatures and eutrophication may contribute to more frequent events and cyanobacterial "super-blooms", with enhanced risks to human health [4]. Recently, *Microcystis* bloom frequently occurred in the eutrophic lakes at Daqing, Heilongjiang Province, China and the dominant species is *Microcystis aeruginosa*, which produces a family of related cyclic heptapeptides (mainly microcystins) [5]. These toxins are severely hepatotoxic, are produced in *Microcystis* cells and are released into water body when cyanobacterial cells are broken.

Microcystin (shown in Fig. 1) is one of over 80 known toxic variants and is the most studied by chemists, pharmacologists, biologists and ecologists. Microcystin-containing "blooms" are a problem worldwide, including China, Brazil, Australia, the United States and much of Europe [6]. Toxic blooms of cyanobacteria have

* Corresponding author. Tel.: +86 021 54341272.
*E-mail address:* dalj@sh163.net (L. Da).

1386-1425/$ - see front matter 
http://dx.doi.org/10.1016/j.saa.2013.09.078

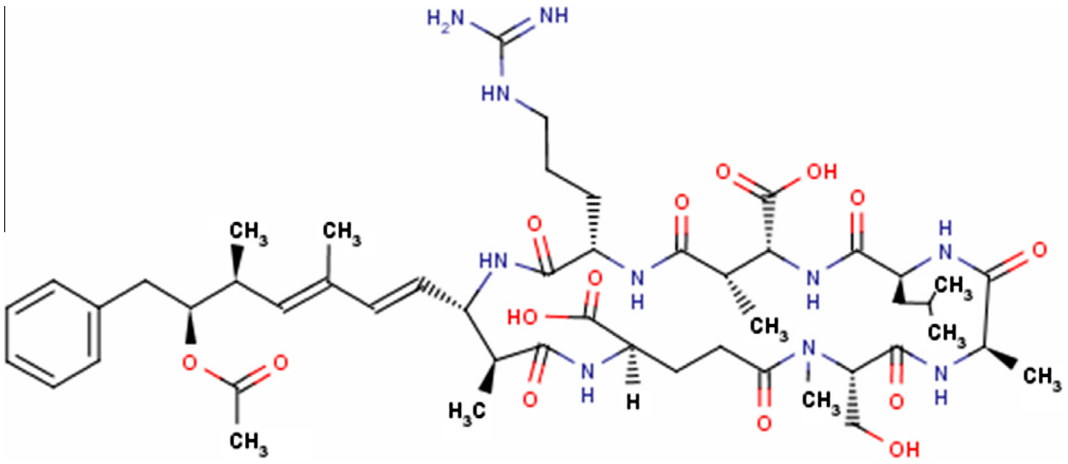


**Fig. 1.** Molecular structure of microcystin.

been associated with acute hepatotoxicity in various species of domestic animals and humans. Once ingested, microcystin travels to the liver, via the bile acid transport system, where most is stored; though some remains in the blood stream and may contaminate tissue [7,8]. Microcystin binds covalently to protein phosphatases thus disrupting cellular control processes. In addition, epidemiologic studies in China have suggested that microcystins in contaminated water may play a role in the higher incidences of primary human hepatocellular carcinomas in many areas, particularly in Daqing, China [5].

Detoxification is one of the liver's functions. Catalase catalyzes the breakdown of hydrogen peroxide, which is toxic (It is highly reactive so can cause cell damage. It comes from the small intestine via the portal vein, from things we eat or drink, e.g. alcohol) to oxygen and water, which are harmless. When the catalase structure is altered and the function is impeded, liver could not work normally. Thus to elucidate the interaction of catalase and microcystin is of great importance to get the insight of the toxic mechanism of microcystin [9–12]. However, there has been no report regarding the toxic effects of microcystin on catalase.

The interaction of microcystin with catalase was investigated under physiological condition in phosphate buffered saline (PBS) buffer solution at pH 7.4 by means of various spectroscopic methods (fluorescence, CD and FT-IR) and enzymatic reactionkinetic technique. Binding constants and the number of binding sites were calculated using Stern–Volmer equations. The thermodynamic parameters are calculated and discussed. The distance $r$ between donor (catalase) and acceptor (microcystin) was obtained according to fluorescence resonance energy transfer and the alterations of catalase secondary structure induced by microcystin were confirmed by 3D fluorescence, FT-IR and CD measurements.

## Materials and methods

Catalase was purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. (Beijing, China). Microcystin with 98% purity was donated by Shanghai Institute of Pharmaceutical Industry, China. All other reagents of analytical grade were used and they were bought from Nanjing Di'an Biotechnology Co., Ltd., China. Double-distilled water was used throughout the experiments. Catalase was dissolved in 0.05 M phosphate buffered saline (PBS) solution to form a $2.0 \times 10^{-6}$ M solution and then preserved at 4 °C for later use.

Steady state fluorescence spectra were acquired on a Hitachi F4010 (Hitachi, Tokyo, Japan) using 1 cm matched quartz cuvettes keeping excitation and emission slit widths of 3 nm. Intrinsic fluorescence of catalase was measured by exciting at 280 nm. Temperature dependent fluorescence spectral studies were performed on the Hitachi unit equipped with a circulating water bath. For Stern–Volmer calculations, the fluorescence data were processed with considerations of inner filter effects [11], which would cause absorption of both excitation and emission radiation. Methods to inner filter effect corrections are taken from Ding [13].

The conformational changes in the protein secondary structures on microcystin binding were studied using a Jasco J815 spectropolarimeter (Shimadzu, Tokyo, Japan) at 25 °C equipped with a Peltier cell holder and temperature controller PFD425 L/15. The protein concentration and path length of the cells used were $2 \times 10^{-7}$ M and 0.1 cm for far UV CD. Secondary structure calculations were performed using the software supplied by Jasco Company.

The absorbance spectra of microcystin in phosphate buffer solution were recorded on a Shimadzu UV2450 spectrophotometer (Shimadzu, Tokyo, Japan). Slit width and scanning speed were set at 2 nm and 100 nm/min, respectively.

FT-IR measurements were carried out on Perkin Elmer Spectrum GX FTIR Spectrometer (Perkin Elmer, Shelton, CT) equipment using ZnSe window. Hundred scans were recorded for each sample in the spectral range of 400–4000 $cm^{-1}$ with a resolution of 4 $cm^{-1}$. The background was corrected before scanning the samples and the buffer spectrum collected. FT-IR spectra of free catalase ($5 \times 10^{-4}$ M) and catalase–microcystin complex (1:2) were recorded to identify changes in secondary structure in catalase on interaction with microcystin. All the experiments were performed at room temperature (25 °C).

The activity of catalase was measured using $H_2O_2$ as the substrate. Catalase can catalyze $H_2O_2$ into water and oxygen. The enzyme activity was obtained based on the decrease in absorbance at 400 nm, where $H_2O_2$ absorbs, in buffered phosphate medium (pH 7.4) containing various concentration of microcystin. One unit of CAT activity is defined as the amount of enzyme that decomposes 1 mmol $H_2O_2$ per minute. The final amount of catalase and $H_2O_2$ are $5 \times 10^{-6}$ M and 8 mM, respectively. The inhibition mechanism was determined using the methods from Lineweaver [14] and the experimental conditions are the same with the activity measurements.

## Results and discussion

### *Fluorescence quenching mechanism of catalase by microcystin*

The fluorescence of protein originates mainly from the amino acid residues of tryptophan (Trp) or tyrosine (Tyr), and