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Study of Interaction between Red-tide Toxin, Domoic Acid and Double –stranded DNA by Capillary Zone Electrophoresis

Da Zhi LI[1], Xin Ya HE[1], Hui WANG[1], Li SUN[2], Bing Cheng LIN[1]*

[1]Dalian Institute of Chemical Physics, Chinese academy of Sciences, Dalian 116023
[2]Dalian Customs Laboratory, Dalian 116001

Abstract: The interactions between amnesic red-tide toxin, domoic acid (DA) and 14mer double-stranded DNA (dsDNA with three kinds of sequences) were studied by capillary zone electrophoresis (CZE). For the dsDNA with a sequence of 5'-CCCCCTATACCCGC-3', the amount of free dsDNA decreases with the increase of added DA; and the signal of DA-dsDNA complex was observed. Meanwhile, the other two dsDNAs, 5'-(C)12GC-3' and 5'-(AT)7-3', the existence of DA could not lead to the change of dsDNA signal and indicated that there is no interaction between DA and these two dsDNAs.

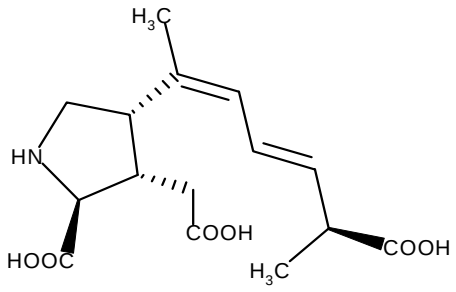
Keywords: DA, dsDNA, interaction, capillary zone electrophoresis.

Contamination of shellfish and fish with natural toxins from the harvest area can cause damage of consumer. Most of these toxins are produced by species of naturally occurring marine algae (phytoplankton). They accumulate in shellfish and some fishes when they feed on the algae, shellfish and other fishes that have fed on the algae[1]. There are four kinds of shellfish poisoning syndromes: amnesic shellfish poisoning (ASP), diarrheic shellfish poisoning (DSP), neurotoxin shellfish poisoning (NSP), and paralytic shellfish poisoning (PSP). DA, an acidic amino acid (**Figure 1**) known as the principal component of ASP, is a kind of water-soluble crystalline compound[2]. The toxic symptoms caused by DA included nausea, disorientation, temporary amnesia and in more serious cases, especially for elder people and /or those with gastric lesions, persistent short-term memory loss and /or coma even death. By now, DA-producing pseudo-nitzschia species have been discovered; their worldwide distribution poses a threat to human health and to the aquaculture industry[3].

DA has become an extensively investigated subject, most of these studies are aimed at the determination of toxins. The trophic transfer of this phycotoxin resulting in mass marine bird and mammal mortality has recently been demonstrated, the receptors of DA binding targets was also found which was predominately to the N-methyl-D-aspartate in the central nervous system[4]. The physiological role of DA to the causative organism is still unknown[5]. Hu *et al.* investigated the interaction between microcystin LR and protein

* E-mail: bclin@dicp.ac.cn

Figure 1 The structure of DA



phosphatase 2A[6]. Rue *et al*. studied the binding of DA with iron and copper[5]. To our knowledge, there is no report concerning the binding of DA directly targeting to DNA. In the present paper, we investigated the interaction between the red-tide biotoxin-DA and dsDNA.

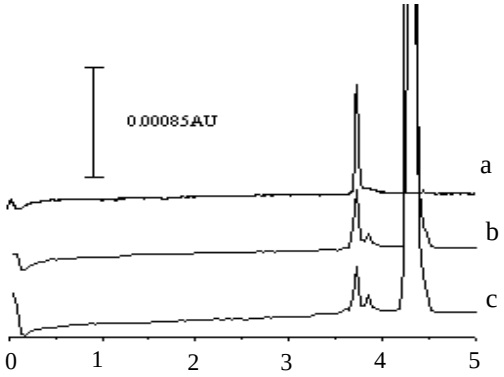
Experimental

DA was purchased from Sigma (St. Louis. MO USA); three different 14-mer dsDNAs, 5'-CCCCCTATACCCGC-3' (sequence 1), 5'-(C)12GC-3' (sequence 2), and 5'-(AT)7-3' (sequence 3) were prepared according to the previous report[7]; redistilled water was used throughout this work; A P/ACE MDQ CE system (Beckman, Fullerton, CA, USA) equipped with a diode array detector (DAD) was used; CE was performed using a polyacrylamide coated capillary (i. d. 50 μm, total and effective lengths were 31.2 and 21 cm, respectively). The conditions for each run were as follows: the temperatures of the cartridge and sample room were 25°C; Samples were injected under 3.45kPa pressure for 4 s, the applied voltage was –9kV, the detection wavelength was 260 nm. After each run, the capillary was rinsed with water for 1 min at 137.89 kPa.

Results and Discussion

The results showed that a dsDNA-DA complex was produced when 14-mer dsDNA with sequence 1 in electropherogram was mixed with DA, meanwhile, the peak height of dsDNA decreased with the increase of DA concentration (**Figure 2**). The existence of the complex under the electric field implies slow on– and – off kinetic binding[8]. In order to probe the interaction selectivity of DA against dsDNA sequence, other sequences of 14-mer dsDNA were mixed with difference concentration of DA, the results showed no interaction between DA with 14-mer dsDNA with sequence 2 (**Figure 3**) and 14-mer dsDNA with sequence 3 (**Figure 4**). Can be observed. Each sample was run in duplication and there was no difference in the results. This is the first report that depicted the binding of shellfish toxin directly to DNA molecule and the preference of shellfish toxin to certain DNA sequence. More studies concerning this type of interaction will be carried out in the further work.

Figure 2 Electropherograms of 19.6 μmol/L14-mer dsDNA with sequence1 mixed with various concentrations of DA



The concentrations of DA were: a) 0 mol/L b) 200 μmol/L c) 400 μmol/L. The applied conditions are described in the experimental section

Figure 3 Electropherograms of 20 μmol/L14-mer dsDNA with sequence 2 mixed with different proportion of DA.

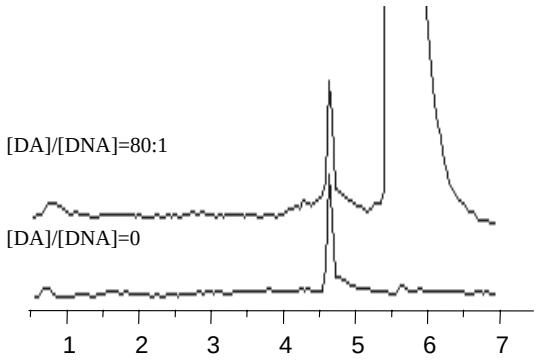
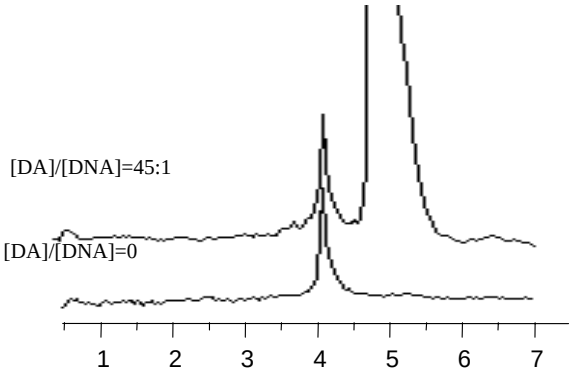


Figure 4 Electropherograms of 20 μmol/L14-merds DNA with sequence 3 mixed with different proportion of DA.



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