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Evaluation of The Interaction between Netropsin and Double Stranded DNA by Capillary Zone Electrophoresis

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Abstract: Capillary zone electrophoresis (CZE) was applied to study the interaction between netropsin and a 14mer double stranded DNA (dsDNA). The binding constant of this interaction calculated from Scatchard plot was $(1.07\pm0.10)\times10^5$ $(mol/L)^{-1}$. The binding stoichiometry was 1:1. The use of polyacrylamide coated capillary showed better effect in the analysis of DNA than noncoated capillary.

Keywords: Interaction, netropsin, DNA, capillary zone electrophoresis.

DNA molecules are polymorphic, and exist in a variety of structural forms that might provide unique binding sites for small molecules[1]. Many small molecules that interact with DNA have potential therapeutic applications in inhibiting tumor growth, and viral, bacterial, fungal and parasitic infections. These drugs can be divided into two categories: intercalator and minor groove binder[2-3]. So DNA-drug interactions are a particularly important class of intermolecular interactions. Many methods have been performed to study the basis of DNA-drug interactions[4]. However, introduction of new methods which are of low consumption of samples and short analysis time is appreciatory to promote the study of DNA-drug interactions.

Capillary electrophoresis (CE) has become a robust separation technique in the past few years owing to several advantages such as short analysis time, low-volume sample requirement, high efficiency and flexible applications[5]. CE is convenient to conduct affinity binding assays by monitoring the changes of the electropherogram signals of the free components and/or the complex. At least five CE methods are served for the characterization of binding interactions[6]: 1) Classical affinity CE; 2) Preequilibration CE (the same as CZE); 3) Hummel-Dreyer principle; 4) Frontal analysis CE; 5) Vacancy peak analysis. Among these methods, CZE is the most appropriate one for the study of molecular interactions in the case very few amounts of samples are available. To our knowledge, no report using coated capillary in CZE for the assay of DNA-drug interactions is available. In this paper, we explored the interaction between a 14-mer dsDNA and netropsin (a minor groove DNA binder) by CZE in a polyacrylamide coated capillary. The signal changes of dsDNA were monitored from the electropherograms of

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the mixtures containing dsDNA and netropsin. The binding constant was obtained based on Scatchard analysis.

Experimental

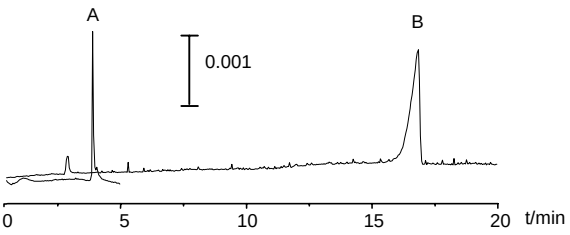
Netropsin dihydrochloride from *Streptomyces netropsis* was from Sigma. The running buffer for CZE was tris-acetate buffer：20mmol/L tris, 10mmol/L NaCl, pH 7.2. Preparation of the 14-mer dsDNA: two complemental single stranded DNAs (ssDNAs) having the sequences of 5′-CCCCCTATACCCGC-3′ and 5′-GCGGGTATAGGGGG-3′ were annealed to form a 14mer oligomer duplex. Redistilled water was used through this work. For the CZE experiments, a P/ACE MDQ system (Beckman, Fullerton, CA, USA) with UV detector was used. The total and effective lengths of the capillary 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 using pressure method with 3.45 kPa for 4 s. The applied voltage was -8 kV and the detection wavelength was 260 nm. After each run, the capillary was rinsed using water for 1 min at 137.89 kPa.

Results and Discussion

Macrobiomolecules may adsorb electrostatically to the surface of fused silica capillaries[7]. This makes the distribution of biomolecules complicated, and thus it is difficult to quantify them. We compared the effects of DNA analysis in a polyacrylamide coated and noncoated capillaries (see **Figure 1**). Apparently, in coated column, peak broadening of DNA is very slight. This is due to that the polyacrylamido- derivative-based inner coating suppressed the adsorption of DNA to the inner capillary wall. The migration time is only 3.89 min compared to 16.82 min in noncoated capillary. Therefore, we prefer coated capillary to noncoated capillary in the binding assay.

The peak height of each sample was proportional to the concentration of dsDNA at the range of 0.5-12.0 μmol/L. The corresponding concentrations of free dsDNA in the binding assay were calculated from this calibration curve: H = 484.9*C + 35.4, r = 0.998.

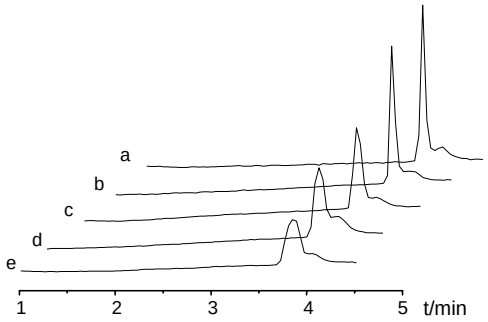
Figure 1 Comparison of the DNA signals in A) coated capillary and B) noncoated capillary.



Conditions: the applied voltages in A and B were -8kV and 8kV, respectively. Other running conditions were identical, see the experimental section.

Figure 2 shows the electropherograms for the mixtures containing the 14-mer dsDNA and netropsin and the 14-mer dsDNA only. The complex no longer survived to reach the detector when we applied an electric field, because dissociation occured on-column. This dissociation process was continuous. This meant that the interaction between netropsin and dsDNA lengthened the dsDNA zone and caused the peak broadening. Accordingly, the peak height of dsDNA decreased (**Figure 2**). This is a common character of the fast on-and-off kinetic binding reaction[8-9]. From the Scatchard plot conducted by using the CE data of various samples, the association constant and the binding stoichiometry were calculated to be $(1.07\pm0.10)\times10^5$ $(mol/L)^{-1}$ and 1:1.

Figure 2 Electropherograms of 11.1 μmol/L 14-mer DNA mixed with various concentrations of netropsin.



The concentrations of netropsin: a) 0 μmol/L b) 3 μmol/L c) 19.8 μmol/L d) 23.8 μmol/L e) 39.9 μmol/L. The applied conditions are as described in the experimental section.

The base specialization that makes netropsin bind preferentially to AT base pairs is provided by close Van der Waals contacts between adenine C-2 hydrogen and CH groups on the pyrrole rings of the drug molecule[10]. But the common characteristics as netropsin is: flat, crescent in shape, positively charged with some donor or acceptor groups, it can fit tightly in the minor groove of dsDNA.

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