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Research Article

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Synthesis and Interaction of YYAAY with CT DNA

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ABSTRACT

A similar ACE inhibitory peptides YYAAY were synthesized by solid phase synthesis. The crude peptide product were separated and analyzed by RP-HPLC, then characterized by ESI-MS. The interaction of YYAAY with DNA had been examined through utilizing fluorescence and ultraviolet spectroscopy and viscosity method. Result showed that the absorption spectra of DNA were decreased with increasing the concentration of YYAAY. When the concentration of DNA increased, the absorption spectra of YYAAY presented hyperchromic effect, and the fluorescence of YYAAY had quenching effect. The viscosity of DNA-YYAAY was decreased too with increasing the concentration of YYAAY. The experimental results revealed that YYAAY inserted in DNA with the mode of the "partial" (or the "nonclassical"). According to the Scatchard equation, the binding constant of YYAAY with DNA was calculated to be $K=2.7\times10^5$ $L\cdot mol^{-1}$

Keywords: similar ACE inhibitory peptide, DNA, interaction, spectroscopy, viscosity method

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INTRODUCTION

In recent years, the hypertensive disease incidence increased rapidly, and developed to the second killer of human health. Angiotensin convert enzyme (ACE) plays an important physiologic role in the blood pressure regulation, ACE inhibitory peptides can effective inhibit it's activity then lower the blood pressure, and has small side effects[1], so ACE inhibitory peptides has been the hotspot in the physiological bioactive peptides. As an important genetic substance of human body, DNA is the main target of anticancer drug [2-6]. Most molecules could affect the DNA replication by interacting with DNA. It is reported that small molecules are bound to DNA double helix by three binding modes: electrostatics binding, groove binding and intercalative binding[7]. so studies on the interactions of ACE inhibitory peptides with DNA are not only importance for clarifying the structure and function of DNA, but also useful for deeper understanding the mechanism of ACE inhibitory peptides' action and designing ACE inhibitory drug.

In this paper, YYAAY which has some structure characteristics of ACE inhibitory peptides was obtained by Fmoc solid phase synthesis[8]. The interaction of YYAAY with DNA had been examined through utilizing fluorescence and ultraviolet spectroscopy and viscosity method. The mode and mechanism of their interaction was discussed preliminary. This paper provided the basic data for the study of peptide and nucleic acids.

EXPERIMENTAL SECTION

2.1. Reagents and methods

2.1.1 Reagents

CT DNA(A.R, Beijing Tiangen Biochem Ltd) was dissolved by pH 7.4 Tris–HCl buffer($c_{(DNA)}$= 2.38×10^{-4}mol·L^{-1});YYAAY was synthesized by Fmoc solid phase and dissolved by pH 7.4 Tris–HCl buffer too($c_{(YYAAY)}$= 2.38×10^{-2}mol·L^{-1});All the other regents are also analytical reagent grade and doubly distilled water

was used throughout the work.

2.1.2 Synthesis and Characterization of YYAAY

According to the references [9], YYAAY was synthesized by Fmoc Solid-phase Synthesis. Firstly, Wang Resin should be put in the synthetic tube and soaked within 10 ml DMF for 30 minutes. Then the Fmoc protecting group of the resin was removed by suspending it in 20% piperidine in DMF solution for 30 minutes. After removed the protecting group, the resin was washed by DMF and methanol, respectively, for three times. Before the amino acids were added to mix for 4-8 hours with N_2 shaking, the resin should be activated by HBTu and HOBt, or DIEA if the acid is difficult to dissolve. When the last amino acid was coupled to the resin, the protecting group was removed in sequence by the same method. The resin was then dried overnight under vacuum. While the dry resin was obtained, the cleavage solution consisting of trifluoroacetic acid (TFA), triisopropylsilane (TLS), and water (9.5 : 0.25 : 0.25), was added and shaken for three hours. The cleavage solution is aliquoted into cold ether by filtration, and solid substance was obtained, washed with cold ether and maintained in the freezer dryer. The product was purified by RP-HPLC and detected by ESI-MS.

RP-HPLC analysis and purification were performed on an Agilent Analytical Semi-prep 1200 HPLC system equipped with a VWD detector. ESI mass spectra were measured on an Esquire 3000 spectrometer from Bruker. UV–VIS spectra were recorded on a Shimadzu UV-2450 spectrophotometer. Fluorescence determination was performed on a Varian F23010 spectrophotometer. Viscosity experiments were carried on an Ubbelohde viscometer, immersed in a thermostated water-bath.

2.2. Experimental Method

2.2.1. Synthesis and Characterization of YYAAY

Firstly, Wang Resin should be put in the synthetic tube and soaked within 10 ml DMF for 30 minutes. Then the Fmoc protecting group of the resin was removed by suspending it in 20% piperidine in DMF solution for 30 minutes. After removed the protecting group, the resin was washed by DMF and methanol, respectively, for three times. Before the amino acids were added to mix for 4-8 hours with N_2 shaking, the resin should be activated by HBTu and HOBt, or DIEA if the acid is difficult to dissolve. When the last amino acid was coupled to the resin, the protecting group was removed in sequence by the same method. The resin was then dried overnight under vacuum. While the dry resin was obtained, the cleavage solution consisting of trifluoroacetic acid (TFA), triisopropylsilane (TLS), and water (9.5 : 0.25 : 0.25), was added and shaken for three hours. The cleavage solution is aliquoted into cold ether by filtration, and solid substance was obtained, washed with cold ether and maintained in the freezer dryer. The product was purified by RP-HPLC and detected by ESI-MS.

2.2.2. Ultraviolet spectra

(1) YYAAY were titrated to DNA

The volume and concentration of DNA were fixed, started ultraviolet spectra scan after YYAAY were titrated to DNA per 5μL, and the same volume and concentration of YYAAY were used as the contrast.

(2) DNA were titrated to YYAAY

The volume and concentration of YYAAY were fixed in series of 10mL comparison tubes, the different volume of DNA were titrated to its according to Rt= C_{YYAAY}/ C_{DNA} = 0, 0.2, 0.4, 0.8, 1, 1.4,2. then added pH 7.4 Tris–HCl buffer to 10ml. UV-Vis absorption spectra were recorded using the same volume and concentration of DNA as the contrast.

2.2.3. Fluorescence spectra

The volume and concentration of YYAAY were fixed in series of 10mL comparison tubes. the different volume of DNA were titrated to its according to Rt= C_{DNA}/ C_{YYAAY} = 0, 0.2, 0.4, 0.8, 1, 1.4, 2, then added pH 7.4 Tris–HCl buffer to 10ml. Fluorescence spectra were carried out. The excitation wavelength and emission of the samples was monitored at 275 and 304 nm.

2.2.4. Viscosity method

The volume and concentration of YYAAY were fixed in Ubbelohde viscometer. Then the different of volume YYAAY were titrated to DNA according to Rt= C_{YYAAY}/ C_{DNA} = 0, 0.2, 0.4, 0.8, 1, 1.4, 2. Data were presented as $(\eta/\eta_0)^{1/3}$ versus Rt([peptide]/[DNA]), where η is the viscosity of DNA in the presence of peptide, and η_0 is the viscosity of DNA alone.

RESULTS AND DISCUSSION

3.1. Synthesis and Characterization of YAGFL

YYAAY was obtained as yellow power in 71% yield by Fmoc solid-phase synthesis. The m/z 650.4 (Fig. 1) matches the molecular ion peak $[M+H]^+$ of YYAAY.

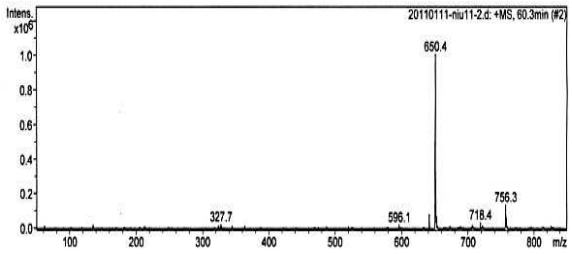

Fig. 1 The ESI-MS spectrum of YYAAY

3.2. Studies on the interaction of YYAAY with DNA by using Ultraviolet spectra

The ultraviolet absorption spectroscopy is a common and convenient method to determine the binding of complexes with DNA.

(1) YYAAY were titrated to DNA

The bases of DNA are the target of drug molecule interaction with DNA, so the position and intensity of the characteristic absorption peak at 260 nm of DNA may change after drug molecule interaction with DNA. Hypochromicity and hyperchromicity are character of DNA spectrum [10]. The hyperchromic effect was caused by the coordination of DNA's bases with drug molecule that changed the double helix structure of DNA. The hypochromic effect was caused by the electrostatic binding or partly intercalation of drug molecule with DNA's bases that twisted the molecule configuration of DNA.

The ultraviolet absorption spectra of DNA is decreased with increasing amounts of YYAAY in PH 7.4 Tris- HCl buffer, but there is no shift at the peak intensities(Figure 2). It is well known that red shift or blue shift at the peak intensities in UV-vis spectra occurred when small molecules interaction with DNA by inlaid mode. The results show that the binding mode of YYAAY with DNA is not the inlaid mode.

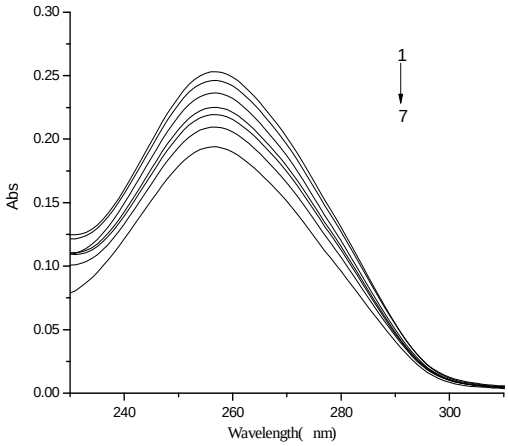

Fig. 2 UV-Vis absorption spectra of different concentration of YYAAY interacted with DNA
$c_{(DNA)}$= 1.19×10^{-5}mol·L^{-1}; $c_{(YYAAY)}$:1~7(0, 0.238, 0.476, 0.952, 1.19, 1.428, 2.38×10^{-5}mol/L)

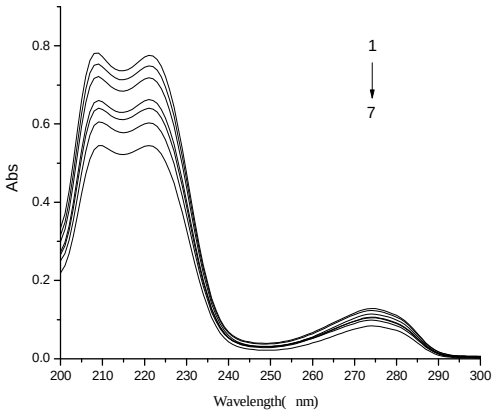

Fig. 3 UV-Vis absorption spectra of different concentration of DNA interacted with YYAAY
$c_{(YYAAY)}$= 1.19×10^{-5}mol·L^{-1};$c_{(DNA)}$:1~7(0, 0.238, 0.476, 0.952, 1.19, 1.428, 2.38×10^{-5}mol/L)

(2) DNA were titrated to YYAAY

The ultraviolet absorption spectra of drug molecule can present hypochromic effect when it insert in the bases of DNA, but when it through electrostatic bind with DNA's bases, it's ultraviolet absorption spectra can present hyperchromic effect [11].From Figure 3,we can see YYAAY has two characteristic absorption peak at 209 nm and 221 nm, and the intensity of the ultraviolet absorption spectra decreased when increased the amounts of DNA, the molecular structure of YYAAY is not absolute planar, so it can't through the inlaid mode bind with DNA. It may partly intercalate in DNA's bases. For further investigations, the interaction of YYAAY with DNA was also studied by using fluorescence spectra.

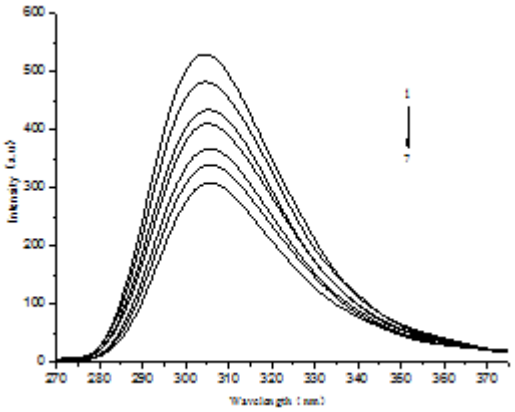

Fig. 4 The fluorescence intensity of different concentration of DNA interacted with YYAAY
$c_{(YYAAY)}$= 1.19×10^{-5}mol·L^{-1}; $c_{(DNA)}$:1~7(0, 0.238, 0.476, 0.952, 1.19, 1.428, 2.38×10^{-5}mol/L)

3.3 Studies on the interaction of YYAAY with DNA by using Fluorescence spectra

YYAAY can emit fluorescence because of its aromatic systems, but DNA can't emit fluorescence. So we can study on the interaction of YYAAY with DNA by the fluorescence quenching of YYAAY through titrating DNA to YYAAY[12].

According to Fig. 4, the fluorescence intensity of YYAAY gets the strongest value at 304nm, and then quenches occurred with increasing amounts of DNA. The results show that DNA has the ability to quench the fluorescence of YYAAY at pH7.4 Tris-HCl buffer, and DNA interacts with the aromatic systems of YYAAY. There are two possible reasons for the decrease of the fluorescence intensity for YYAAY[13]: Firstly, complex which has no fluorescence is produced when DNA and YYAAY are at ground state. Secondly, excited molecules of DNA and YYAAY collide with each other. There are two quenching process- static quenching and dynamic quenching [14].

If the quenching belongs to dynamic quenching, it follows Stern-Volmer equation: $F_0/F = 1 + K_q\tau_0[Q]$

Where F and F_0 are the fluorescence intensities before and after the addition of DNA, respectively. K represents the quenching constant and [Q] is the concentration of DNA. The values of F_0/F were plotted against [DNA] (Fig.5).

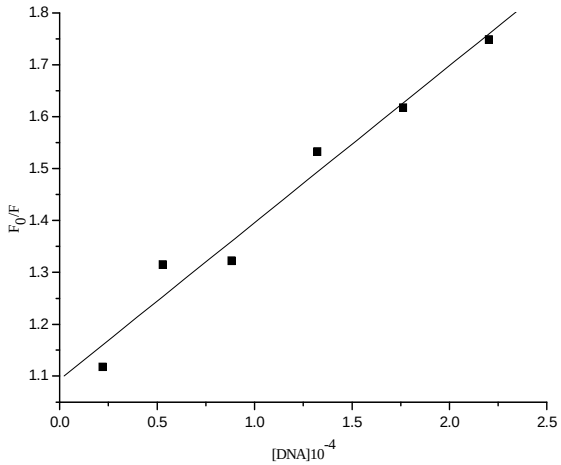

Fig. 5 The values of F_0 / F at different concentration of DNA

The quenching constant K of the interaction between DNA and YYAAY is 5.3×10^{11} L·mol^{-1}, which is much bigger than the biggest collision constant between small molecular and biological macromolecules 2×10^{10} L·mol^{-1}. Therefore, the quenching belongs to static quenching, it can be expressed by Lineweaver-Burk equation: $(F_0 - F)^{-1} = F_0^{-1} + K_D^{-1} F_0^{-1} [DNA]^{-1}$. The values of$(F_0\text{-}F)^{-1}$were plotted against $[DNA]^{-1}$(Fig. 5). we can obtain the K of the interaction between DNA and YYAAY is 2.7×10^5 L·mol^{-1} calculated by Lineweaver-Burk equation(Fig.6).

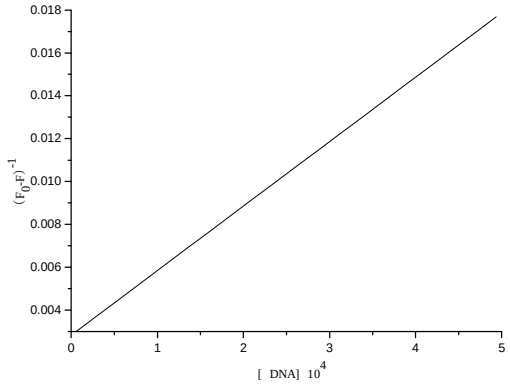

Fig. 6 Lineweaver-Burk plot of the interaction between YYAAY and DNA

3.4 Studies on the interaction of YYAAY with DNA by using Viscosity method

Spectroscopic data are necessary, but not sufficient to support a binding mode. So as a means for further clarifying the binding of peptide with DNA, viscosity measurements were carried out on DNA by varying the concentration of the added peptide. A classical intercalative mode causes a significant increase in viscosity of DNA solution due to increase in separation of base pairs at intercalation sites and hence an increase in overall DNA length. On the contrary, when drug molecule partial (nonclassical) intercalates in DNA, the viscosity of DNA solution decreases, which dues to the double helix structure of DNA is twisted. Under the same conditions, there are less pronounced (positive or negative) or no change in DNA solution viscosity when they bind by electricity[15,16].

The values of $(\eta / \eta_0)^{1/3}$ were plotted against [peptide]/[DNA] (Fig.7). The relative viscosities of DNA decrease obviously (Fig.6). The result indicates that YYAAY partially intercalate in DNA.

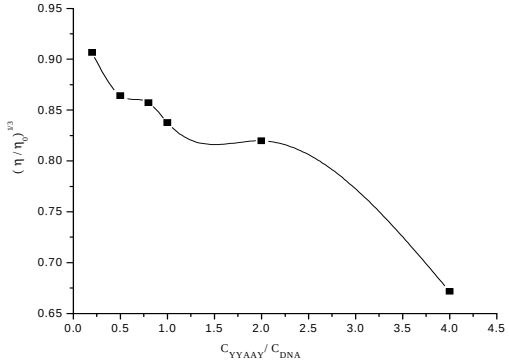

Fig. 7 The relative viscosity of DNA in different concentrations of YYAAY
$c_{(DNA)}$= 1..428×10^{-5}mol·L^{-1}; $c_{(YYAAY)}$:1~7(0, 0.267, 0.714, 1.142, 1.428, 2.856, 5.721×10^{-5}mol/L)

CONCLUSION

YYAAY was obtained as yellow power in 71% yield by Fmoc solid-phase synthesis and characterized by ESI-MS: m/Z 650.4 $[M+H]^+$. Fluorescence spectroscopy, ultraviolet spectroscopy and viscosity method are carried out in this work to study the interaction of YYAAY with DNA. The results showed that the YYAAY could interact with DNA by mainly partial intercalative mode. The K of the interaction between DNA and YYAAY was 2.7×10^5 L·mol^{-1}.

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