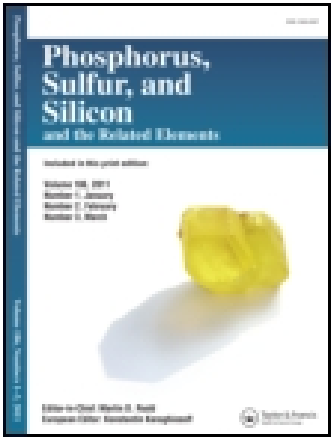

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Investigation on Interaction of L-Methionine Dipeptide with ct-DNA by Ultraviolet Spectroscopy

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In this article, L-methionine peptides were synthesized with the assistance of phosphorus oxychloride. L-methionine dipeptide was purified by HPLC and characterized by the means of ^{1}H-NMR, ^{13}C-NMR and ESI-MS. The interaction of L-Methionine dipeptide with ct-DNA was studied by ultraviolet spectra. The results showed that L-Methionine dipeptide could interact with phosphorous groups of ct-DNA. The influences of interaction time, ionic strength, and phosphate anion on the interaction between L-methionine dipeptide and ct-DNA were also investigated.

Keywords ct-DNA; interaction; *L*-methionine dipeptide; Phosphorus oxychloride; ultraviolet spectra

INTRODUCTION

The study of small molecules that specific sites along a DNA helix has become a subject of considerable interest.[1–7] Several small molecules have been shown to interact with DNA at the molecular level by specific binding modes. These binding studies were driven partly by the need to understand the mechanism of anticancer drug action,[8] to decipher the chemical basis for the carcinogenicity of environmental pollutants and toxic chemicals[9] and to serve as analogues in studies of protein-nucleic acid recognition,[10] provide site specific reagents for molecular biology, and yield rationales for new drug design. Many small molecules based chemical reagents have already been proved to be useful as sensitive probes of local nucleic acid structure.

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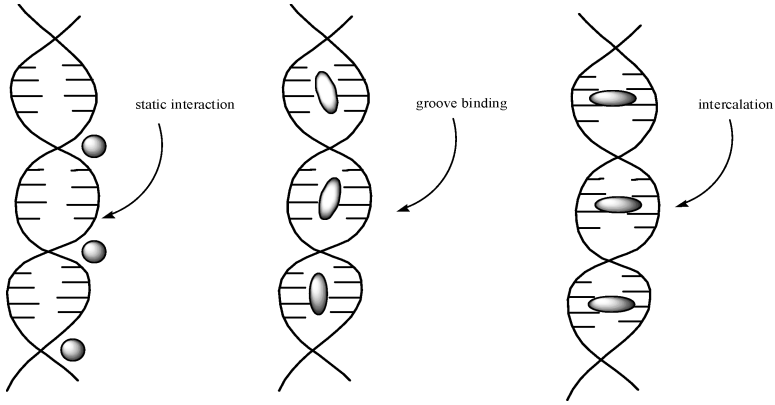

FIGURE 1 Illustration of the static interaction, intercalation and groove binding of small molecules to DNA.

Small molecules interact with the DNA double helix by three dominant modes, namely static interaction, intercalation and groove binding (Figure 1). It would be valuable to understand quantitatively the contributions from these different modes to the stabilization of bound DNA complex.

Phosphorus plays an important role in the origin of life and control of life; we studied the peptide formation reactions with the assistance of inorganic phosphorous compound.[11] The investigation on interactions between biological macromolecules and small molecules by ultraviolet spectroscopy is common, sensitive and convenient.[12] In this article, we prepared *L*-methionine dipeptide (Met-Met) by assembled reaction with the assistance of phosphorus oxychloride, and explored the interaction of Met-Met with calf thymus DNA (ct-DNA) by ultraviolet spectroscopy. The result of ultraviolet absorption spectra suggested the static interaction between Met-Met and ct-DNA.

EXPERIMENTAL

Reagents and Chemicals

L-Methionine was purchased from Yuanju Biochemical Co. (Shanghai, China). Phosphorus oxychloride, acetonitrile and THF (HPLC grade) were purchased from Tianjin Chemical Reagent Co. (Tianjin, China). Calf thymus DNA (ct-DNA) (Sigma Co., America) was commercially purchased and accepted for use without further purification. The stock solutions were prepared by dissolving the solid DNA in doubly distilled water and stored at 4°C. The concentration of DNA was determined

according to the absorbance at 260 nm after establishing that the absorbance ratio A260/A280 was in the range of 1.80–1.90. The molarities of DNA were calculated using $\varepsilon_{DNA} = 6600\ M^{-1}cm^{-1}$.[13] The stock solution of 5.6×10^{-3} mol L^{-1} Met-Met (Prepared and purified by HPLC) was prepared by directly dissolving its crystal into water. A sodium acetate-acetic acid buffer solution (pH 4.8) was used to control the pH value of the interacting system. Deionized water was generated from Milli-Q water purifying system purchased from Millipore (MA, America). Other common chemicals used were of the highest purity commercially available.

Apparatus

A Bruker Esquire 3000 plus ion trap mass spectrometer interfaced to electrospray ionization (ESI) source was used for mass analysis and detection. Agilent 1200 HPLC system consisted of a UV detector, a Ternary HPLC pump with a **SB** C18 prepared column (10 μm, 250 mm $\times$ 10 mm). Peaks were monitored by UV absorbance at 214 nm. A mixture of acetonitrile (A) and water (B) was used as the mobile phase. The gradient elution was programmed as follows: 0–5 min, 70% B; 5–15 min, 70–80% B; 15–20 min, 85% B; 20–25 min, 85–70% B, and 25–30 min, 70% B. The column temperature was maintained at room temperature (25 °C). The flow rate was 1.0 mL/min. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker DPX-400 spectrometer, using $CDCl_3$ as solvent and TMS as internal standard. IR spectra were taken from Shimadzu IR-Prestige 21 spectrophotometer. The ultraviolet absorption spectra were recorded on a TU-1900 spectrophotometer.

Preparation of L-Methionine Dipeptide

L-Methionine oligopeptide libraries were prepared according to the literature,[11] and the mixture of oligopeptides was separated and prepared on **SB** C18 prepared column (10 μm, 250 mm $\times$ 10 mm) using a mixture of acetonitrile (A) and water (B) as elute. Then, the collection was concentrated under reduced pressure. *L*-Methionine dipeptide was obtained as white power in 4.01% yield (225 mg). m.p.:148–150°C; IR (KBr pellet, cm^{-1}): 3440, 3330, 2950, 2920,1660, 1580, 1450, 1380, 1420, 1340; ^{1}H-NMR ($CDCl_3$, ppm): δ1.97 (s, 3H, CH_3), 1.98 (s, 3H, CH_3), 1.87–2.07 (m, 4H, $2CH_2$), 2.40–2.54 (m, 4H, $2CH_2$), 4.03 (t, $J = 6.2$ Hz, 1H, CH), 4.32 (t, $J = 6.1$Hz, 1H, CH); ^{13}C-NMR ($CDCl_3$, ppm): δ 13.88 (SCH_3), 27.81 (CH_2S), 29.97 (CH**C**H_2CH_2), 52.07 (HN**C**HCO), 53.01 (H_2N**C**HCO), 168.92 (HN**C**OCH), 175.98 (CH**C**OOH); MS^2, *m/z* (RA%): 264.2 (19), 233.3 (7), 187.4 (3), 150.5 (100), 132.6 (<1), 104.8 (52).

Spectral Measurements

The ultraviolet absorption titrations with DNA were conducted by keeping the concentration of nucleic acid constant, and varying the Met-Met concentrations. This was done by dissolving an appropriate amount of Met-Met in the DNA stock solution and mixing various proportions of Met-Met and the DNA stock solutions, while maintaining the total volume of the solution constant. This resulted in a series of solutions with varying concentrations of Met-Met but with a constant concentration of DNA. The ultraviolet absorbance of the samples was monitored at 260 nm. The temperature (37°C) of the solution was continuous monitored with a thermo-couple attached to the sample holder.

The intrinsic binding constants of Met-Met with calf thymus DNA was determined by ultraviolet absorption. In the case of the former, the absorbance at 260 nm was recorded after each addition of Met-Met. The intrinsic binding constant K was determined from Eq. (1):[14]

$$A_0/A = -(1 - A/A_0)\, cK + npK + 1, \tag{1}$$

where K is the intrinsic binding constant; n is the binding site size; p is the concentration of DNA determined by the absorbance at 260 nm; c is the concentration of Met-Met. A; and A_0 are the absorbance values of DNA-Met-Met and DNA at 260 nm, respectively. The binding data were treated by constructive methods and values of K and n were obtained.

The effects of interaction time, ion strength, and phosphate ion on the interaction of Met-Met and DNA were investigated by controlling interaction time, and varying ion strength, phosphate and ion concentration of the interaction system.

RESULTS AND DISCUSSION

Study of Ultraviolet Absorption Spectra

The ultraviolet absorption spectra of DNA was decreased with increasing amounts of Met-Met (hypochromicity), but there is no obvious shift at the peak intensities (Figure 2). The absorbance of DNA in the peak (260 nm) was decreased by *ca.* 20%. The addition of further amounts of Met-Met to the DNA solution did not cause any more absorption changes, due to the binding saturation of the Met-Met with DNA. We considered the electrostatic interactions between the Met-Met and DNA because of the dipeptide with positive charge and DNA with negative polyphosphate skeleton. Hypochromism was suggested to be due to weak interactions between the electronic states of the

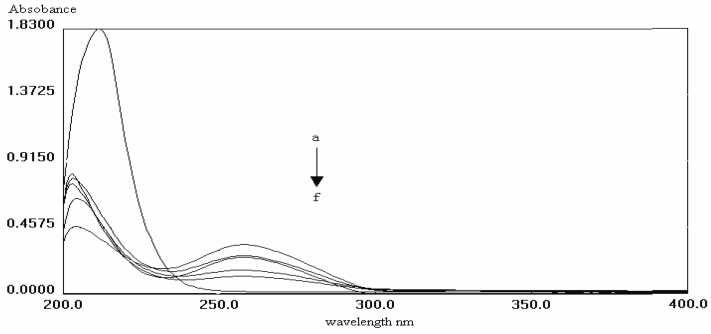

FIGURE 2 The change of ultraviolet spectra of dipeptide-DNA solutions with dipeptide concentration R = [Met-Met]/[DNA]; a: R = 0; b: R = 1; c: R = 1.5; d: R = 3.0; e: R = 5.0; f: [DNA] = 0.0.

dipeptide and that of the DNA phosphate ion. The observed spectral changes (hypochromicity) are consistent with the static interaction between dipeptide and DNA phosphate ion.

Absorbance data of DNA at 260 nm with different concentration of Met-Met were used to obtain the intrinsic binding constant by Equation (1) (Figure 3). According to Rosenthan plot, the best-fit linear regression equation was: $A_0/A = -0.711 + 0.722\,(1-A/A_0)\,c$ (R = 0.9982). Then, the intrinsic binding constant of Met-Met with DNA (Ka) of 1.39×10^2 L/mol was deduced.

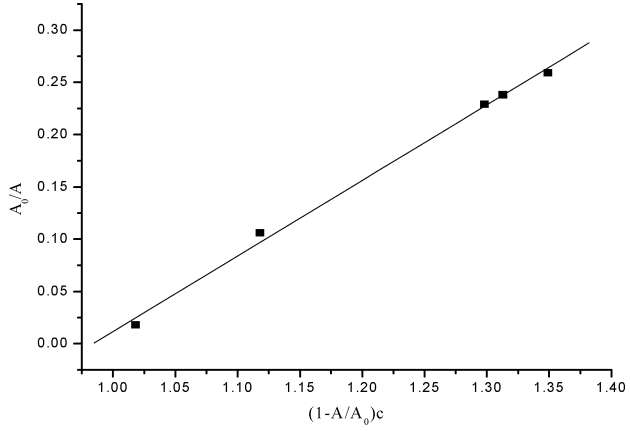

FIGURE 3 Rosenthan plot of DNA (5.6×10^{-5} mol/L) with Met-Met dipeptide as determined from the electronic absorption spectra.

The Effects of Interaction Time, Ion Strength and Phosphate Anion on the Interaction System

It was expected that the longer the interaction time of L-methionine dipeptide with ct-DNA, the more the hypochromic effect. It was found, however, that there was an energetic equilibrium after 3 h.

Moreover, in order to test whether the electrostatic interaction between Met-Met and DNA exists, the strong electrolyte, sodium chloride (NaCl), was used. The electrostatic interaction would be weakened by the addition of counter ion because of the conversion of the electrostatic atmosphere of DNA periphery. Some reports showed that very high concentration of NaCl would hinder the binding of small molecule with DNA.[15] If there did exist electrostatic interaction between Met-Met and DNA, by increasing the ionic strength in the solution of Met-Met and DNA, the absorbance of solutions would increase when NaCl was added, but it would never exceed the absorbance of the pure DNA solution. However, the effect was not as great as expected. The ultraviolet absorption were decreased greatly from 0.360 to 0.310 when the concentration of NaCl (i.e., ionic strength) increased from 0.00 to 0.10, but there was no obvious change for ultraviolet absorption when the concentration of NaCl increased further (Figure 4), which indicated that there was competition of interaction between Met-Met and NaCl with DNA. It is suggested that the additional sodium cations interacted with DNA, and hindered the interaction of Met-Met with DNA. So the probable intercalation process was that Met-Met was attracted to the periphery of DNA by electrostatic attraction. If other cations occupied the effective range of electrostatic interaction, the affinity of Met-Met with DNA would be greatly weakened. Anyway, the salt effect could not inhibit the binding of Met-Met with DNA completely.

Furthermore, the electrostatic interaction between Met-Met and DNA was tested by the influence of phosphate anion (Table I). As shown in Table I, the observed spectral changes (hypochromicity) were weakened with adding phosphate anion, which indicated that Met-Met was attracted to the periphery of DNA by electrostatic attraction.

TABLE I Influence of Phosphate Anion on the Binding of Met-Met with DNA

KH_2PO_4 Concentration	0	0.005	0.01	0.02	0.05	0.1	0.2	0.5
A_{260}	0.360	0.332	0.335	0.337	0.341	0.333	0.323	0.313
δA_{260}	0	0.028	0.025	0.023	0.019	0.027	0.037	0.047

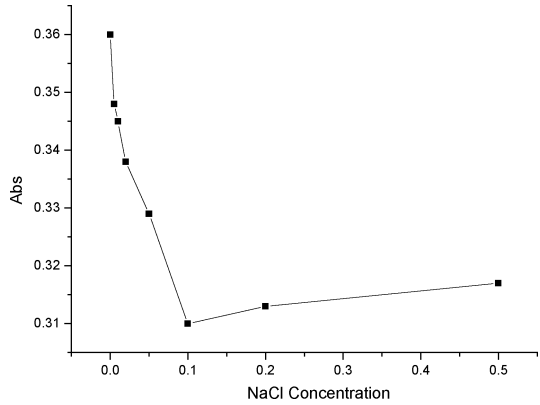

FIGURE 4 Influence of ionic strength on the binding of Met-Met with DNA.

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