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RESEARCH ARTICLE

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Study on the interaction of trypsin with some DNAs and their analytical application by resonance Rayleigh scattering spectra

Abstract When trypsin reacts with Herring sperm DNA (hsDNA), Salmon sperm DNA (sDNA), and Calf thymus DNA (ctDNA) to form a complex, the resonance Rayleigh scattering (RRS) was remarkably enhanced and new RRS spectra appear. These new spectra have similar characteristics of RRS spectra. The maximum RRS peaks are at 307 nm (hsDNA, sDNA) and 290 nm (ctDNA), and other peaks are at 350 nm. The scattering intensity is proportional to the concentration of DNA or trypsin; so this intereaction can be used to determine trypsin using DNA or DNA using trypsin. In the determination of DNA using trypsin, the linear ranges for hsDNA, sDNA, and ctDNA are 0–2.3, 0–2.5, and 0–1.9 $\mu g \cdot mL^{-1}$, and the detection limits are 0.4, 0.7, and 1.1 $ng \cdot mL^{-1}$, respectively. In the determination of trypsin using hsDNA, the linear range is 0–30.0 $\mu g \cdot mL^{-1}$, and the detection limit is 39.0 $ng \cdot mL^{-1}$. In this paper, the intereaction conditions were optimized. The affecting factors, chemical properties of the complex, and the composition ratio of trypsin with DNA were investigated. Using trypsin as RRS probe, a sensitive method for the determination of trace amounts of DNA was developed.

Keywords resonance Rayleigh scattering, trypsin, hsDNA, sDNA, ctDNA

1 Introduction

The interaction between protein and DNA plays a key role in many life processes [1]. The molecular recognition of protein and DNA is very significant in the expression of a gene and its regulation mechanics [2]. It is also important to realize the process and the essence of the interaction between these two kinds of biomacromolecules to research the binding mode and express it at molecular level [1–4]. At present, X ray diffraction analysis is the most direct method to get the information of structural model of complexes [2,5,6]. It is very difficult to separate, purify, and prepare the crystal of Protein–DNA complexes, and some reactions cannot produce a stable crystal, which limits the types of reaction. In addition, nuclear magnetic resonance [7,8] and mass spectrum [9,10] are also often used for this purpose. The common molecular spectral methods, such as spectrophotometry, fluorophotometry, and chemiluminence, are seldom used in this field. One reason is that the interaction between proteins and DNA usually cannot result in obvious change in the absorption and fluorescence spectrum and can seldom produce chemiluminence and bioluminence, and the other reason is that the reaction between proteins and DNA is very complicated and particular. Therefore, the above spectral methods are hard to afford definite information. Li et al. [11] investigated the interaction between protamine sulfate and DNA using nephelometric method, but the sensitivity was not high (the detection limit of ctDNA is 12.5 $ng \cdot mL^{-1}$), and the information afforded was very limited.

The resonance Rayleigh scattering (RRS) has a sharp response to the electrostatic attraction, hydrophobic force, hydrogen bond, and aggregation of molecules. The spectral characteristics and scattering intensity can be affected by molecular size, shape, and conformation. This method has a high sensitivity and simplicity and can be used to research the interaction of biomacromolecules [12,14].

In this paper, the characteristics of RRS for the interactions of human serum albumin (HSA), bovine serum albumin (BSA), pepsin, papain, and trypsin with calf thymus DNA (ctDNA), herring sperm DNA (hsDNA), and salmon sperm DNA (sDNA) were investigated The results showed that the interaction of trypsin with DNA can result in the greater enhancement of RRS [1,2]. Therefore, using trypsin–DNA system as an example, the RRS characteristics, the optimum interaction conditions, and the binding ratio were studied.

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2 Experimental

2.1 Apparatus and reagents

A Hitachi F-2500 spectrofluorophotometer (Tokyo, Japan) with 1-cm quartz cells was used for recording RRS spectra and measuring RRS intensities. The slit is 5.0 nm/5.0 nm (EX/EM). A UV–VIS 8500 spectrophotometer (Tianmei, Shanghai) was used for recording the absorption spectra and a PHS-3C meter (Shanghai Precision, Scientific instrument Co., Ltd.) was used for measuring pH.

Nucleic acid (NA) solution: hsDNA (Sigma Co.), sDNA(Sigma Co.), and ctDNA(Sigma Co.) were dissolved in 0.5% NaCl solution. The concentrations of NAs are determined according to the absorbance at 260 nm after establishing that the absorbance ratio A_{260}/A_{280} is in the range 1.8–1.9. The concentration of the stock solution of DNA is 20 $\mu g \cdot mL^{-1}$. The concentration of the stock solution of trypsin (Sigma Co.) is 1 $mg \cdot mL^{-1}$. Both protein and DNA solutions are kept at 1–4°C.

Britton—Robinson (BR) buffer solutions (pH 2–6) were prepared by mixing 0.04 $mol \cdot L^{-1}$ H_3PO_4, H_3BO_3, HAc and 0.2 $mol \cdot L^{-1}$ NaOH in proportion, and their pH values were corrected by pH meter.

Double distilled water was used in all cases. Proteins and NAs are all biochemical reagents. All other reagents were of analytical grade.

2.2 Experimental procedure

BR solution (2.0 mL), suitable amount of DNA solution, and 1.0 mL of trypsin solution were added into a 10.0-mL volumetric flask. The resulting solution was diluted to 10.0 mL with distilled water and then mixed thoroughly. The RRS spectra of the systems were recorded after 10 min, with synchronous scanning at EX = EM. RRS intensities for the complexes (I_{RRS}) and the reagent blank (I_0) were measured at the maximum wavelength ($\Delta I_{RRS} = I_{RRS} - I_0$).

3 Results and discussion

3.1 Characteristics of RRS spectra

Figure 1(A) shows the RRS spectra of trypsin-DNA systems. It can be seen from Fig. 1(A) that the RRS intensities of trypsin, hsDNA, sDNA, and ctDNA are very weak. When trypsin reacts with DNAs to form the complexes, the RRS intensities are enhanced greatly and new spectra can be observed. The spectral characteristics of the complexes are similar. All the maximum peaks are at 307 nm and the other smaller peaks are at 350 nm. The maximum RRS peaks are chosen as the detection wavelength. The sensitivities of the four reaction systems are in the order of hsDNA > sDNA > ctDNA. Figure 1(B) shows the RRS spectra of trypsin–hsDNA system and the RRS intensities are proportional to the concentration of hsDNA.

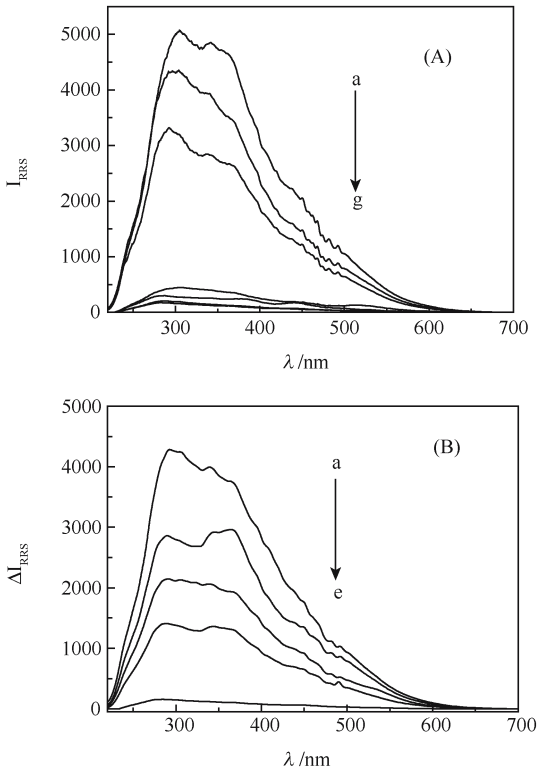

Fig. 1 RRS spectra of trypsin-DNA system (**A**) and trypsin-hsDNA system (**B**)
(**A**) a, hsDNA–trypsin; b, ctDNA–trypsin; c, sDNA–trypsin; d, trypsin; e, ctDNA; f, hsDNA; g, sDNA; ρ (DNA) = 1.24 $\mu g \cdot mL^{-1}$; ρ (trypsin) = 100 $\mu g \cdot mL^{-1}$. (**B**) a–d,. hsDNA–trypsin; e, trypsin; ρ. (hsDNA) /($\mu g \cdot mL^{-1}$): a, 1.90; b, 1.14; d, 0.38; c, 0.76

3.2 Optimum reaction conditions

3.2.1 Effect of acidity

Effect of pH on absorbance is shown in Fig. 2. According to Fig. 2, the optimum pH ranges of the three DNA–trypsin systems are 2.2–3.0 (hsDNA), 2.2–4.0 (sDNA) and 3.0–4.7 (ctDNA). We chose pH 2.6 for hsDNA, sDNA systems and pH 4.3 for ctDNA system.

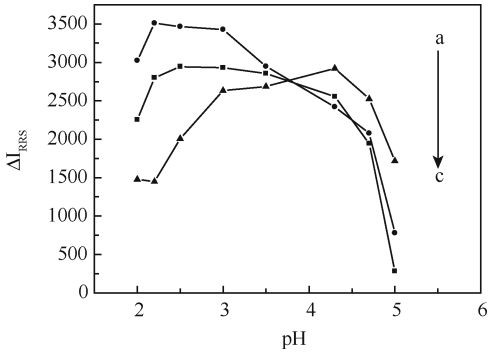

Fig. 2 Effect of pH on RRS intensity
a, hsDNA–trypsin; b, sDNA–trypsin; c, ctDNA–trypsin.
ρ (DNA) = 1.1 $\mu g \cdot mL^{-1}$; ρ (trypsin) = 100 $\mu g\ mL^{-1}$

3.2.2 Reaction time and the stability of RRS intensity

The reaction time and stability of RRS were also tested. The results show that the reactions can complete in 10 min (hsDNA), 10 min (sDNA), and 20 min (ctDNA). The absorbance can be stable for 12 h.

3.2.3 Effect of the concentration of trypsin on the RRS intensity

The effect of the concentration of trypsin on the RRS intensity is shown in Fig. 3. It shows that the RRS intensities increase with the increase in the concentration of trypsin until the concentration reaches 150 $g \cdot mL^{-1}$ (hsDNA, sDNA) or 100 $g \cdot mL^{-1}$ (ctDNA). Therefore, both the concentrations of trypsin were chosen for the reaction.

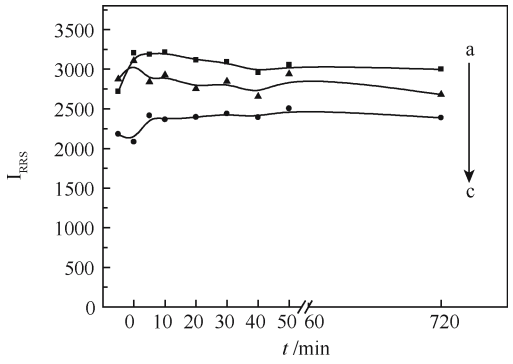

Fig. 3 Stability of RRS intensity
a, hsDNA–trypsin; b, sDNA–trypsin; c, ctDNA–trypsin.
ρ (DNA) = 1.1 $\mu g \cdot mL^{-1}$; ρ (trypsin) = 100 $\mu g\ mL^{-1}$

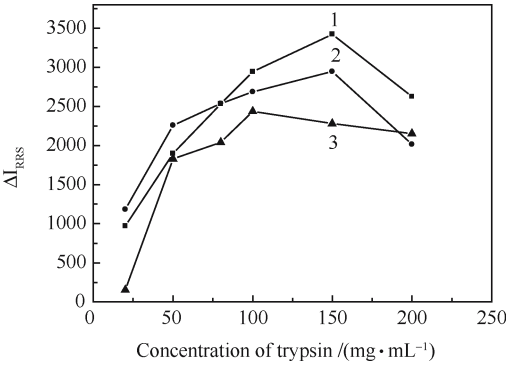

Fig. 4 Effect of concentration of trypsin
1, hsDNA—trypsin; 2, sDNA–trypsin; 3, ctDNA–trypsin.
ρ (DNA) = 1.0 $\mu g \cdot mL^{-1}$

3.2.4 Standard curve and the detection limits

Under optimum conditions, ΔI_{RRS} values are plotted against the concentration of NAs. All the parameters of the standard curves and detect limits are listed in Table 1. The linear ranges are 0–2.3 $\mu g \cdot mL^{-1}$ (hsDNA), 0–2.5 $\mu g \cdot mL^{-1}$ (sDNA), and 0–1.9 $\mu g \cdot mL^{-1}$ (ctDNA), and the detection limits are 0.4 $ng \cdot mL^{-1}$ (hsDNA), 0.7 $ng \cdot mL^{-1}$ (sDNA), and 1.1 $ng \cdot mL^{-1}$ (ctDNA). The sensitivity of this method is higher than that of common fluorophotometry (as shown in Table 2). We can also determine the concentration of protein using DNA as the reagent by RRS. For example, the linear range of trypsin is 0.1–30.0 $\mu g \cdot mL^{-1}$ and the detection limit is 39.0 $ng \cdot mL^{-1}$.

Table 1 Some parameters of the calibration graph and the detection limits for DNA

DNA	Linear range /($\mu g \cdot mL^{-1}$)	Linear regression equation	Correlation coeffcient	Detection limit /($ng \cdot mL^{-1}$)
hsDNA	1.4×10^{-3}–2.3	$\Delta I = -390 + 4272.3c$	0.9973	0.4
sDNA	2.1×10^{-3}–2.5	$\Delta I = 382 + 2832.9c$	0.9993	0.7
ctDNA	3.5×10^{-3}–1.9	$\Delta I = 307 + 1851.48c$	0.9973	1.1

3.3 Interaction of trypsin with DNA and analysis of RRS enhancement

3.3.1 The binding effect of trypsin with DNA

The composition ratio of trypsin with DNA is 1:1, which is determined by the Job's method and molar ratio method. In other words, a mole of trypsin can bind to a mole of DNA to form complexes. The binding constants are 0.17 (hsDNA), 0.13 (sDNA), and 0.12 (ctDNA).

Although it is hard to judge the binding mode and the reaction model only according to RRS spectra of the binding products, it can infer the common reasons for the interaction. Because trypsin can react with the selected DNA, the interaction is nonspecific. This kind of reaction is caused by salt bridge formed by the electrostatic attraction between the negatively charged DNA and the positively charged proteins below its isoelectric point (pI of trypsin is 10.5), which is an ideal model of nonspecific interactions of proteins with NAs [1]. Therefore, the value of pI influences the RRS response of proteins and DNA to some extent, namely, at the same acidity. A higher pI value indicates that there are more positive charges on proteins and hence there is a stronger electrostatic attraction for DNA. Among the selected proteins, the pI value of trypsin is highest; therefore, at the same condition, trypsin system has a higher RRS response. In addition, the hydrogen bond effect, the hydrophobic interaction, and the aromatic ring stacking between NA base pair and side chain of protein may also be the important reasons for their binding reaction.

3.3.2 Reasons of resonance enhancement

(1) Increase of the volume of the binding products

The increase of volume of the complex is an important reason for RRS enhancement. When it is hard to calculate the volume of the complex, we can use the molecular weight to measure. Trypsin is a small protein (the molecular weight is 23,300), and the molecular weight of common DNA is larger

Table 2 Comparison of the sensitivities for DNA of this method with some fluorescence and RRS methods

Method	Reagent	Measurement wavelength $\lambda_{ex}/\lambda_{em}$/(nm)	Linear range/(μg · mL^{-1})	Determination limit/(ng · mL^{-1})	Ref.
F	Tb(III)-phen	298 (543.5)	0.4–15	100	3
			0.4–20	200	
F	Eu(III)-phen	398 (615)	0.02–1.0	10	4
F	Ethidium bromide	540 (590)	0.01–10.0	25	5
F	Nile blue	627 (672)	0.003–2.0	3.0 (ctDNA)	6
F	Phosphin 3R	460 (505)	0.1–0.2	5 (ctDNA)	7
RRS	TAAP	432 (432)	1.8–10.8	13.53	8
RRS	Safranine T	350 (350)	0–14.8	13.2 (ctDNA)	9
			0–19.8	39.8 (fsDNA)	
RRS	Methylene blue	355 (355)	0–1 .4	11 (ctDNA)	10
		560 (560)	0–1.2	4 .9 (fsDNA)	10
RRS	Neutral red	330 (330)	0.048–5.23	48.2	11
RRS	Ethyl violet	510 (510)	0–0.5	1.54	12
RRS	Crystal violet	495 (495)	0–0.9	5.02	13
RRS	Histone	551 (551)	0–2.0	3.0	14
RRS	Trypsin	307 (307)	0–1.9	0.42 (hsDNA)	Present work
			0–2.48	0.65 (sDNA)	
			0–2.3	1.06 (ctDNA)	

F, fluorophotometry; RRS, resonance Rayleigh scattering method
phen, *o*-phenanthroline; TAAP, tetrakis[4-(trimethylammoniumyl) phenyl] porphine

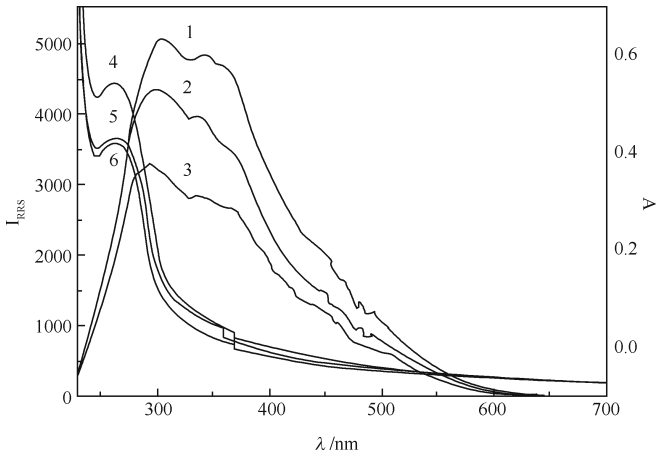

Fig. 5 Comparison of spectral characteristics of absorption with RRS for trypsin-DNA complex
(1) RRS spectrum of trypsin-hsDNA; (2) RRS spectrum of trypsin-sDNA; (3) RRS spectrum of trypsin-ctDNA; (4) absorption spectrum of trypsin-sDNA; (5) absorption spectrum of trypsin-hsDNA; (6) absorption spectrum of trypsin–ctDNA

than that of trypsin and is in the range of (10^7–10^8) [29]. When trypsin binds with DNA to form 1:1 complexes, the molecular weights increase remarkably. According to the simplified Rayleigh formula [30]:

$$I = KI_0Mc$$

When the intensity of incident light (I_0), concentration of DNA (c), and the determining condition are constant, the scattering intensity (I) is proportional to the molecular weight. So, the increase of molecular weight can cause the enhancement of RRS signal.

(2) Resonance enhancement effect

The RRS is an elastic scattering with the scattering wavelength equaling the incident wavelength. When the size of the scattering particle is far smaller than the wavelength of the incident light, the scattering is Rayleigh scattering. The diameter of trypsin is only several nanometers and that of DNA is also smaller; when they bind with each other to form a 1:1 complex, the diameter of the binding product will be less than 10 nm. The area of the binding interface of DNA-protein complexes reported is between 112 and 580 nm, and the diameter of the complex is calculated to be between 6 and 26 nm, according to the calculation formula of area of a round, which is far smaller than the wavelength of the incident light [2]. Therefore, this elastic scattering is thought as Rayleigh

Table 3 Effect of coexisting substance

Coexisting substance	Coexisting times	Coexisting substance	Coexisting times
DL-Aspartic acid	1.8	Ba (II)	9.6
DL-Threonine	9.0	Hg (II)	134.2
L-Isoleucine	16.4	Fe (III)	0.5
L-Tyrosine	6.4	NH_4 (I)	6.2
DL-Tryptophane	22.7	Cd (II)	3.4
Adenine	18.2	Pb (II)	6.1
Starch	20.0	Mn (II)	2.1
Sucrose	4.6	Zn (II)	8.5
Lactose	22.7	Co (II)	5.2
Glucose	45.5	Ca (II)	8.2
Ni (II)	2.0	Cu (II)	5.4
K (II)	17.8	Urea	31.8
Al (III)	0.5	SDS	15
CO_3^{2-}	7.5	Egg albumin	10.9
BSA	2.7	HSA	3.2

Table 4 Results for the determination of hsDNA in synthetic samples

Sample	hsDNA/(μg·mL^{-1})	Coexisting substance and their concentrations /(μg·mL^{-1})	Determined concentration /(μg·mL^{-1})	Recovery/(%)	RSD ($n=5$)
1	0.90	Sucrose 1, lactose 3, Ca(II) 2.5, D,L-tryptophane acid 2, L- isoleucine acid 2	0.88	97	8.2
2	0.90	Aspartic acid 1, BSA 2, adenine 2, Fe (III) 1, CO_3^{2-} 1	0.89	98	9.7
3	0.90	NH_4 (I) 1, HSA 1, Ca (II) 2.5, starch 5, urea 3	0.87	96	5.8

scattering. When it is located at its absorption band, the Rayleigh scattering can absorb the energy of the light to produce an absorption-rescattering process, which can enhance the intensity of scattering remarkably. This process is called as the resonance enhanced effect [31,32].

According to Fig. 5, the RRS spectra of trypsin with the three DNA systems are all located at their absorption bands, and the maximum scattering wavelength (290 or 307 nm) is close to the maximum absorption wavelength (260 nm), which can cause a resonance enhanced effect to increase the intensity of RRS remarkably.

3.4 Selectivity of the method

3.4.1 Effect of coexisting substance

Taking hsDNA as an example, influences of coexisting substance within $\pm 5\%$ relative error have been investigated, and the results are shown in Table 3. According to Table 3, when the concentration of hsDNA is 1.1 μg·mL^{-1}, several times than 1.1 μg·mL^{-1} of Ca (II), Mg (II), Fe (II), Ba (II), Zn (II), Co (II), Cd (II), and Ni (II) are tolerable. The permitted amounts of Fe (III) and Al (III) are little. Several times, even dozens of times, of amino acids, urine, adenine, SDS, some proteins, and some other saccharides (starch, sucrose, lactose, glucose) are also tolerable. In brief, most common coexisting substance has little influence on the spectra of trypsin-hsDNA system. This method has good selectivity.

3.4.2 Determination of proteins in synthetic samples

Three synthetic samples with different concentrations of hsDNA were determined. The results are shown in Table 4.

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