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Studies on interaction between CdTe quantum dots and α-chymotrypsin by molecular spectroscopy

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Abstract. In this article, the interaction between α-Chymotrypsin and CdTe QDs was investigated by fluorescence, synchronous fluorescence, and circular dichroism (CD) spectroscopic methods at pH 7·20 and pH 9·05. The intrinsic fluorescence of α-Chy is quenched by CdTe QDs. Under different pH conditions, the level of binding constants is determined to be 10^3 from fluorescence data. The hydrogen bond or van der Waals force is involved in the binding process when pH is 9·05, while the hydrophobic and electrostatic interactions play main role in the binding process when pH is 7·20. The red-shift of synchronous fluorescence spectral peak of protein after the addition of CdTe QDs reveals that the microenvironments around tryptophan residues are disturbed by CdTe QDs. The secondary structure of α-Chy undergoes slight changes as similar by far-UV CD data. The activity and stability of α-Chy in the presence of CdTe QDs were also studied. α-Chy can maintain its high activity and stability under different pH conditions for 24 h in the presence of CdTe QDs.

Keywords. CdTe QDs; α-chymotrypsin; interaction; fluorescence spectroscopy; CD spectroscopy; enzyme activity.

1. Introduction

Luminescent semiconductor quantum dots (QDs), also called nanocrystals (NCs), have attracted increasing attention in the past decade.[1] Because of quantum confinement, the QDs possess unique optical properties, such as tunable PL, high photostability, long luminescence lifetime and attractive spectrum with narrow emission and broad excitation.[1] Because of these properties, the use of QDs has been demonstrated in biology and medicine as fluorescent probes and more recently in analytical chemistry.[2–7] Colloidal semiconductor CdTe QDs, in particular, are of interest due to their large Bohr radius (7·3 nm) and the relatively small band gap (1·475 eV), leading to a pronounced quantum size effect in a range of CdTe QDs with a diameter up to 10 nm. In addition, the absorption and emission spectra of these QDs can reach the near-IR spectral regime. CdTe QDs are nanoscale spherical particles that have the potential to overcome some of the functional limitations encountered by organic dyes in fluorescence labelling applications.[8] Recently, mesoporous sllica beads or polystyrene beads doped with multicolour QDs have been used for biological applications, including DNA detection, multiplexed bioassays, and immunoassays of immunoglobulin G (IgG); however, the studies have been rather limited.[9–10]

It is well known that enzymes are mild, highly specific and efficient biocatalysts in a wide range of applications.[11–13] α-Chymotrypsin (α-Chy) is one of the most studied enzymes and its structure and mechanism of action are well-known.[14] The α-Chy is a globular β protein with 245 amino acids and a secondary structure dominant in anti-parallel β-sheet with a small α-helix content that has a catalytic activity in the hydrolysis of ingested proteins in the intestine.[15] The activity of the enzyme is modified by the presence of lipid/water interfaces and extensive studies have been reported regarding the characteristics of esters (such as 2-naphthylacetate) and

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amides (*N*-glutaryl-l-phenylalanine *p*-nitroanilide) hydrolysis in reverse micellar solutions[16] and aqueous surfactant solutions.[17] It is well-known that α-Chy, and as other proteases such as, protein kinases and phosphatases, possesses a broad selectivity toward their substrates; α-Chy substrates can be divided into 'poor' and 'good' substrate, depending on their kinetic parameters, especially kcat/K_M; this 'specificity constant' has been properly used to obtain information about enzyme–substrate specificity.[18]

In this study, the applicability of the CdTe QDs modified by thioglycolic acid to the labelling of α-chymotrypsin (α-Chy) was investigated by a spectroscopic method. Fluorescence spectroscopy was employed to understand the quenching mechanism of CdTe-Chy. Effects of CdTe QDs on conformational changes of α-Chy were investigated by synchronous fluorescence spectroscopy and circular dichroism (CD). The binding constants have been determined and the binding mode inferred from the thermodynamic parameters.

2. Experimental

2.1 *Materials*

A standard solution of α-Chy containing 2 mg/ml (8×10^{-5} M) was prepared. *N*-acetyl-L-tyrosine ethyl ester (ATEE) and α-Chy were obtained from Chinese Drug and Biological Product Detection Institute. All other chemicals used were analytical grade reagents. Doubly distilled water was used for the preparation of all solutions.

2.2 *Preparation of NaHTe*

NaHTe solution was prepared according to the reported method with minor modifications.[19] Deoxygenized water (4 ml) was added to $NaBH_4$ (0·275 g) held in a small flask. After the solution was cooled with ice-water, tellurium powder (0·220 g) was added with continuous stirring. The stirring was continued for 8 h until all of the tellurium powder was dissolved. The final solution was used for further experiments.

2.3 *Preparation of CdTe QDs*

CdTe QDs were prepared using the reported method with minor modifications.[20] $CdCl_2 \cdot 2{\cdot}5H_2O$ (0·2854 g) was dissolved in 280 ml of water, thioglycolic acid (0·22 ml), was added under stirring and the pH was adjusted to 11·20 by drop-wise addition of 0·1 M solution of NaOH. The solution was placed in a three-necked flask fitted with a septum, deaerated by bubbling N_2 for about 30 min. Under stirring, 1·50 ml of freshly prepared oxygen-free NaHTe solution was added to the solution rapidly. The resulting mixture was heated under reflux at 100°C for 3 h. The final concentration of QDs was $4{\cdot}5 \times 10^{-3}$ M (referring to as Cd^{2+} here in after) and was stored at 4°C.

2.4 *Apparatus and methods*

Transmission electron micrographs (TEM) of CdTe QDs were performed on JEM-2100F (200 kV). In spectroscopic experiments, the α-Chy solution was introduced into the CdTe QDs colloid at a certain concentration. The incubation time was about 2·5 h in order to attain binding equilibrium. Fluorescence spectra were measured with a LS-55 (Perkin-Elmer, USA) spectrofluorimeter equipped with a xenon lamp, 1 cm quartz cell, using 8 nm/8 nm slit widths. The excitation wavelength was 280 nm, and the emission was recorded at 290–450 nm. 0·1 ml of the $8{\cdot}0 \times 10^{-5}$ M α-Chy solution and an appropriate volume of the CdTe QDs colloid solution were placed into a 5 ml volumetric flask. The mixture was diluted to 5 ml with physiological buffer. All experiments were measured at four temperatures (278, 290, 298 and 308 K). The data were analysed by using the Stern–Volmer equation to calculate the binding constants.

The synchronous fluorescence spectra were recorded by scanning simultaneously the excitation and emission wavelength at 15 or 60 nm intervals.

Circular dichroism (CD) measurements were carried out on a Jasco J-810 sepectrophotometer using a 1 cm quartz cell. The CD spectra of α-Chy solutions containing CdTe QDs were recorded from 190 to 300 nm. Corresponding absorbance contributions to CdTe QDs colloid and buffer were also measured and were subtracted from those for the test solutions.

For the measurement of α-Chy activity, *N*-acetyl-L-tyrosine ethyl ester (ATEE) was used; the change in absorbance at 237 nm was followed in the reaction mixture (2·8 ml) containing 5 mM physiological buffer (pH 7·20 or pH 9·05) and 1 mM ATEE. The reactions were initiated by adding 0·2 ml of 2 mg/ml enzyme.[21]

3. Results and discussion

3.1 *Fluorescence studies of α-Chy quenched by CdTe QDs*

Figure 1 shows transmission electron microscopy (TEM) images of the CdTe QDs. The morphology and size of CdTe QDs could be observed clearly. The particles appear spherical and no aggregation was observed. The average size of studied CdTe QDs was about 3–4 nm, and considered close to the value resulting from the empirical formula which seems to be convenient to calculate the size of CdTe QDs.[22]

The interaction of CdTe with α-Chy at different pH conditions was evaluated by monitoring the intrinsic fluorescence intensity changes of α-Chy upon addition of CdTe. Fluorescence quenching spectra of α-Chy at the presence of various concentrations of CdTe are shown in figure 2. Fluorescence of α-Chy originates from tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe) residues. Actually, α-Chy has eight tryptophan (Trp) residues. The intrinsic fluorescence of α-Chy is mainly contributed by the Trp residue alone, because the Phe residue has a very low quantum yield and the fluorescence of Tyr is almost totally quenched when it is ionized or when it is near an amino group, a carboxyl group or a Trp.[23] The change of intrinsic fluorescence intensity of α-Chy is mainly generated by Trp residue when small molecule substances are bound to α-Chy. As illustrated in figure 2, the fluorescence spectra of enzyme upon excitation at 280 nm at pH 7·20 and 9·05 show an identical emission maximum at 350 nm, which is characteristic of partial shielding of the tryptophan residues from aqueous solvent.[24]

The addition of CdTe QDs resulted in a distinct red shift in the wavelength maximum and a corresponding decrease in the fluorescence intensity, implying that the binding of CdTe QDs to α-Chy occurs and the microenvironment around chromospheres of α-Chy is changed. Furthermore, the change of α-Chy fluorescence at two pH values is

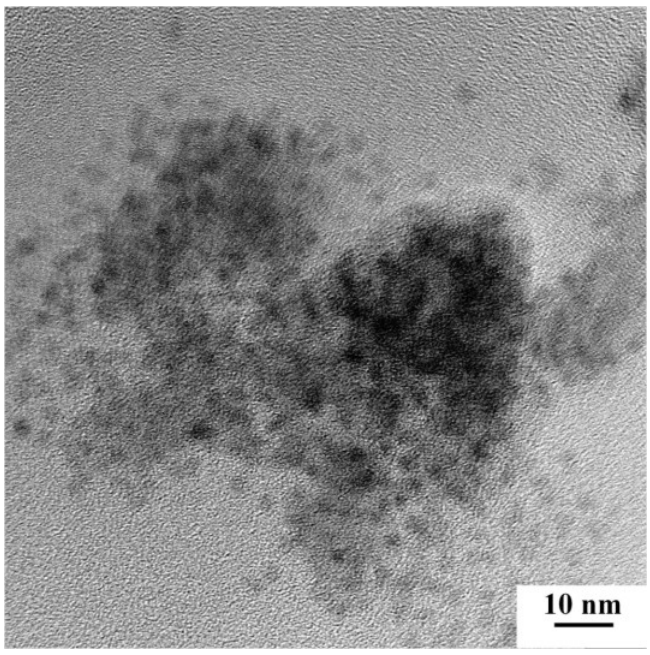

Figure 1. The TEM graph of CdTe QDs.

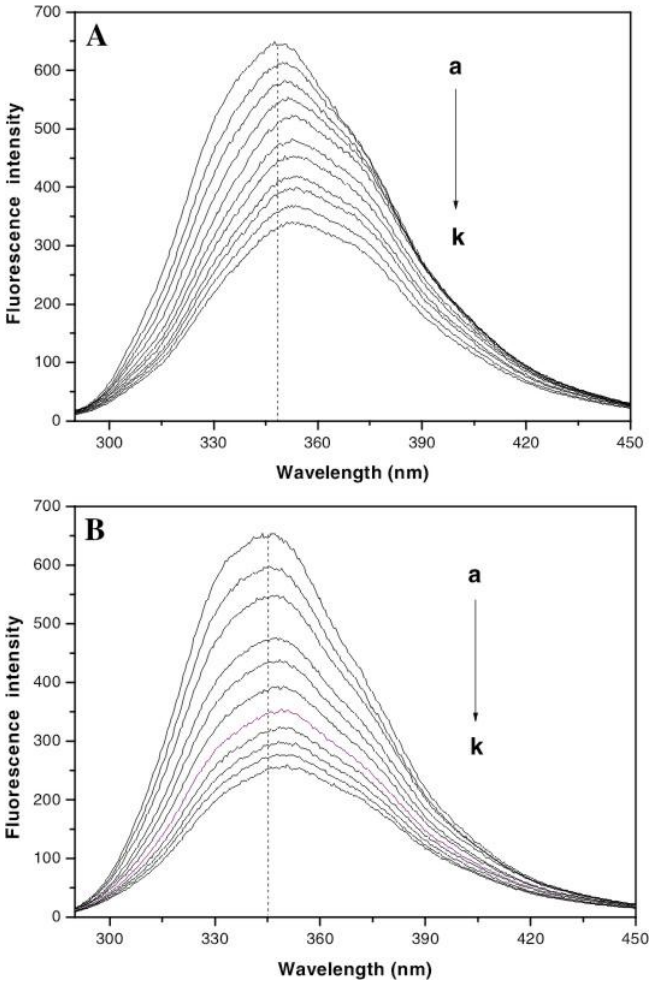

Figure 2. Fluorescence spectra of α-Chy in the absence and presence of CdTe QDs. The concentration of α-Chy was fixed as $1{\cdot}6 \times 10^{-6}$ M; the concentrations of CdTe QDs from (**a**) to (**k**) were 0, 0·6, 1·20, 1·80, 2·40, 3·00, 3·60, 4·20, 4·80, $5{\cdot}40 \times 10^{-5}$ and $6{\cdot}00 \times 10^{-5}$ M, respectively; (**A**) pH = 9·05, (**B**) pH = 7·20.

different. At pH 9·05, under similar concentration of QDs, the emission maximum exhibits larger red shift and smaller decrease in the fluorescence intensity. This result indicates that the Try residues of α-Chy under these conditions is more exposed in the polar environment. The observed fluorescence quenching probably arises from the energy transfer occurring between α-Chy and CdTe QD, which will be proved in following results.

Fluorescence intensity data were then analysed according to Stern–Volmer quenching equation.[25]

$$\frac{F_0}{F} = 1 + k_q \tau_0 [Q] = 1 + K_{SV}[Q]. \quad (1)$$

Where F_0 and F are the steady state fluorescence intensities of α-Chy at 348 nm before and after the addition of quencher (CdTe), *Ksv* and $[Q]$ are the Stern–Volmer dynamic quenching constant and the concentration of quencher CdTe, respectively, kq is the quenching rate constant of biomolecule, τ_0 is the average life-time of bimolecular without the quencher and its value is 5 ns.[26] The solid lines in figure 3 show the lines of best fit of the experimental data to the Stern–Volmer equation. The values for K_{SV}, k_q, R, and linear regression equation at different temperatures are presented in table 1. The results in table 1 show K_{SV} is inversely correlated with temperature, which suggests that the fluorescence quenching process may be mainly controlled by a static quenching mechanism rather than a dynamic quenching mechanism. Moreover, values for k_q (table 1) are two orders of magnitude greater than the maximum diffusion collision quenching rate constant ($2{\cdot}0 \times 10^{10}$ L/mol/s) of a variety of quenchers with biopolymer.[27] This result indicates again that the quenching is not caused by dynamic collision but from the formation of a complex.

For the static quenching process, the quenching data were analysed according to the modified Stern–Volmer equation.[28]

$$\frac{F_0}{\Delta F} = \frac{1}{f_a} + \frac{1}{f_a K_A [Q]}. \quad (2)$$

In the present case, F_0 and ΔF are the relative fluorescence intensity without quencher and the difference in fluorescence intensity of protein in the absence and presence of quencher, respectively. K_A is the effective quenching constant for the accessible fluorophores, here it is binding constant and $[Q]$ is the quencher concentration; f_a is the fractional maximum fluorescence intensity of protein summed up, and the plot of $F_0/\Delta F$ vs $[Q]^{-1}$ is linear with slope equaling to the value of $(f_a K_A)^{-1}$. The value $1/f_a$ is fixed on the ordinate. The association constant K_a is a quotient of an ordinate $1/f_a$ and slope $(f_a K_A)^{-1}$.

Figure 4 shows the modified Stern–Volmer plots for the system of CdTe-Chy at different temperatures. The corresponding results at different temperatures are listed in table 2.

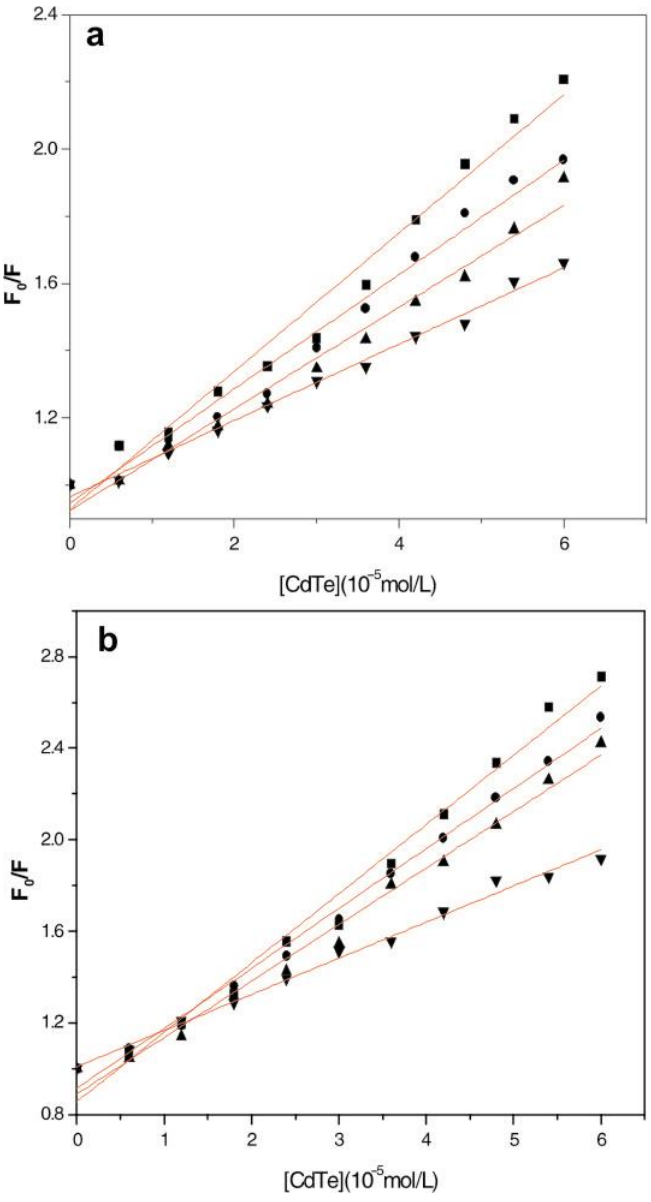

Figure 3. The Stern–Volmer plots of α-Chy-CdTe QDs system. ■ 278 K; • 290 K; ▲ 298 K; ▼ 308 K. [α-Chy] = $1{\cdot}6 \times 10^{-6}$ M, λex = 280 nm; (**a**) pH = 9·05, (**b**) pH = 7·20.

Table 1. Stern–Volmer quenching constants of CdTe–α-Chy system at different temperatures.

pH	T(K)	K_{sv} ($\times 10^4$ L/mol)	K_q ($\times 10^{12}$ L/mol/s)	R
9·05	278	2·14	4·28	0·9892
	290	1·76	3·52	0·9889
	298	1·60	3·20	0·9933
	308	0·73	1·46	0·9913
7·20	278	3·19	6·38	0·9952
	290	2·72	5·44	0·9986
	298	2·59	5·18	0·9968
	308	1·57	3·14	0·9952

Table 2. Binding constants (K) and thermodynamic parameters for α-Chy binding to CdTe.

pH	Temperature (K)	K_A (10^3 L mol^{-1})	ΔG^0 (kJ mol^{-1})	ΔH^0 (kJ mol^{-1})	ΔS^0 (J mol^{-1} K^{-1})
9·05	278	4·65	–19·54	–39·04	–70·13
	290	2·50	–18·70		
	298	1·48	–18·14		
	308	1·09	–17·44		
7·20	278	7·09	–20·49	8·71	104·99
	290	8·15	–21·71		
	298	8·97	–22·55		
	308	10·25	–23·15		

Table 2 shows that the order of magnitude of K is 10^3. Luminescent titration experiments have shown that straight, A-tract, and 'kinky' DNA binding to CdS nanoparticles has the binding constants of 4×10^2, 5×10^2 and 3×10^3 M^{-1}, respectively.[29] In contrast, in the present study, the binding constants of α-Chy to CdTe QDs are higher than those of DNA molecules. Such a high value of K explains the strong affinity of CdTe QDs surface to α-Chy. At the same time, it can be seen that the binding of CdTe to α-Chy at pH 7·20 is stronger than that at pH 9·05 (table 2).

3.2 *Thermodynamic parameters and binding mode*

The thermodynamic parameters were analysed in order to characterize further the binding mode of α-Chy to CdTe QDs. Basically, four types of interactions play vital roles in protein binding. These are hydrogen bonds, van der Waals forces, electrostatic forces, and hydrophobic interactions. Thermodynamic parameters, free energy changes (ΔG^0) enthalpy changes (ΔH^0) and entropy changes (ΔS^0) of the interactions are essential to interpret the binding mode.

Values of ΔG^0, ΔH^0, and ΔS^0 calculated from Vant Hoff plot of ln$K$ versus $1/T$ (see figure 5) are listed in table 2. Negative values of ΔG^0 reveal that the binding process is spontaneous under two pH values. When pH values are 7·20, a positive value of ΔS^0 along with a lesser ΔH^0 value is characteristic of electrostatic interactions in aqueous solution.[30] Moreover, positive values of ΔS^0 provide evidence of hydrophobic interactions. Thus, it is more likely that hydrophobic and electrostatic interactions are involved in binding of α-Chy and QDs under pH 7·20. When the pH value is 9·05, a negative value of ΔS^0 along with a negative ΔH^0 value is characteristic of hydrogen bonds or van der Waals forces in aqueous solution.[30] The isoelectric point (pI) of α-Chy is in the pH range of 8·10–8·40. Therefore, an electrostatic interaction contributes to the binding of α-Chy to the thioglycolic acid-rich surface of CdTe QDs at pH 7·20. Hydrogen bonds or van der Waals forces play a main role in the binding of α-Chy to surface of CdTe QDs at pH 9·05. Furthermore, any conformational changes of the protein invariably result in

entropic gain under two pH values,[31] which may be one of the important driving forces for α-Chy binding to the surface of CdTe QDs.

3.3 *Energy transfer between α-Chy and CdTe QDs*

According to the Förster's theory,[32] the energy transfer effect is not only related to the distance between the donor (tryptophan residue) and acceptor (CdTe QDs), but also influenced by the critical energy transfer distance R_0. By Förster's theory, the efficiency of energy transfer (E) is calculated using the equation:

$$E = \frac{R_0^6}{R_0^6 + r^6} \tag{3}$$

where r is the distance between the acceptor (CdTe) and the donor (α-Chy), and R_0 is the critical energy transfer distance, at which 50% of the excitation energy is transferred to the acceptor is defined by the following equation:

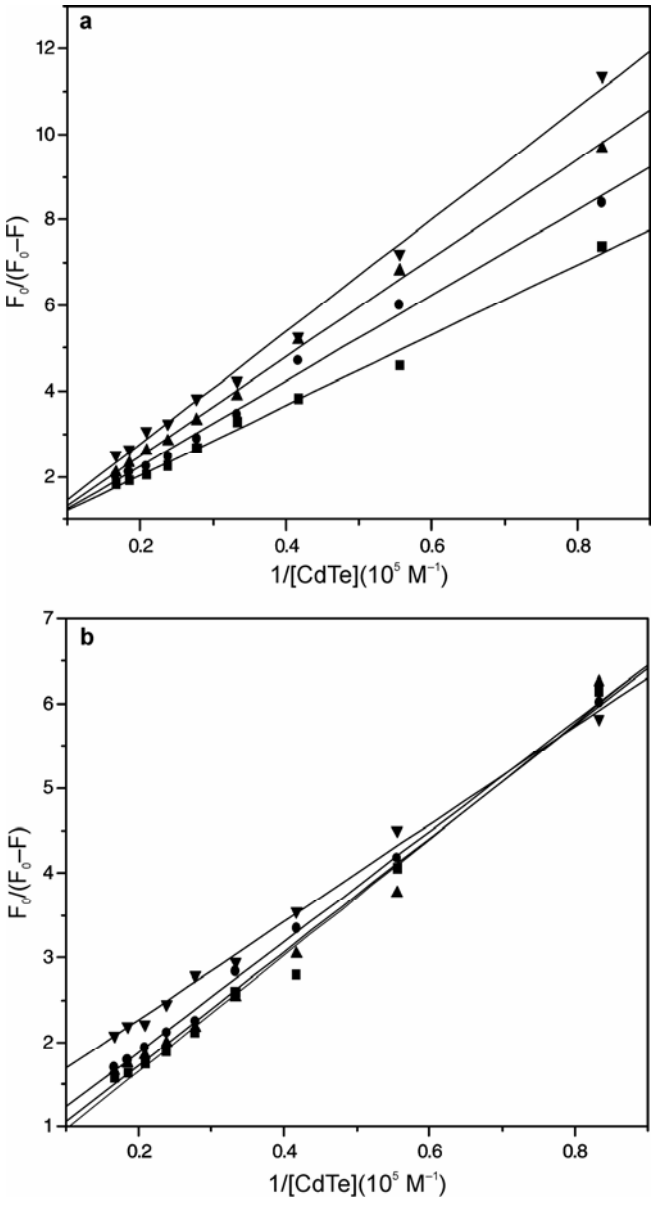

Figure 4. The modified Stern–Volmer plots for the CdTe-Chy system. ■ 278 K; • 290 K; ▲ 298 K; ▼ 308 K. [α-Chy] = 1·6 × 10^{-6} M, λex = 280 nm; (**a**) pH = 9·05, (**b**) pH = 7·20.

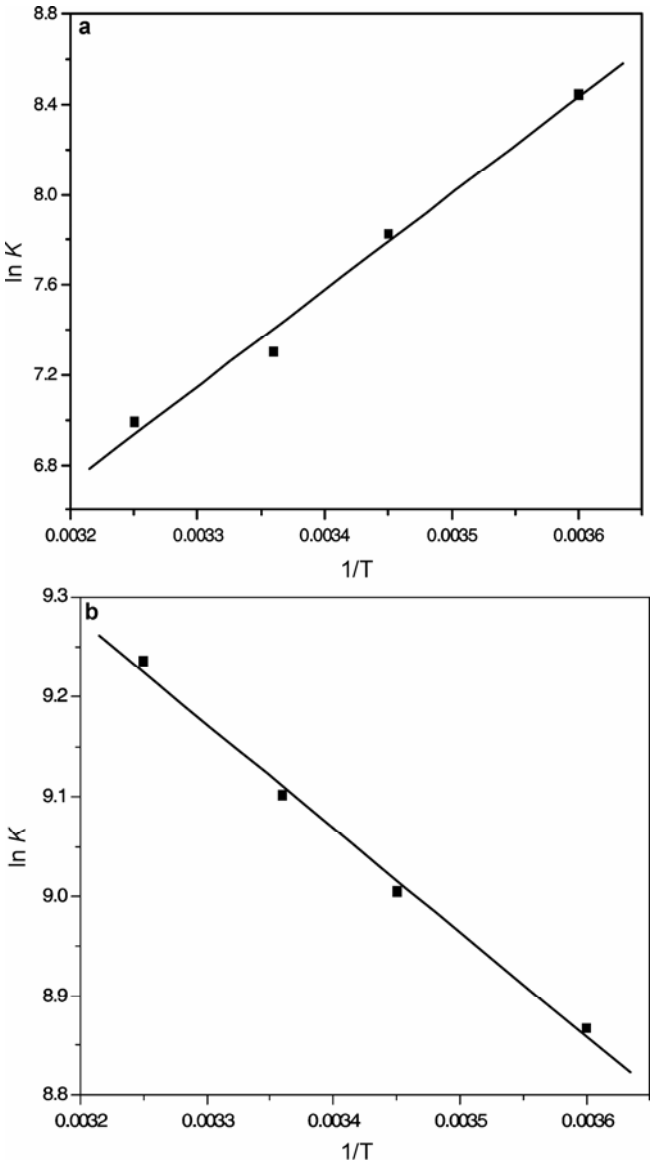

Figure 5. Vant Hoff plot, [α-Chy] = 1·6 × 10^{-6} M; (**a**) pH = 9·05, (**b**) pH = 7·20.

$$R_0^6 = 8{\cdot}8 \times 10^{-25} k^2 N^{-4} \Phi J. \quad (4)$$

In eq. (6), k^2 is the spatial orientation factor of the dipole, N is the refractive index of medium, Φ is the fluorescence quantum yield of donor, and J is the spectral overlap between the emission spectrum of donor and the absorption spectrum of acceptor (figure 6), which is given by:

$$J = \frac{\sum F(\lambda)\varepsilon(\lambda)\lambda^4 \Delta\lambda}{\sum F(\lambda)\Delta\lambda}. \quad (5)$$

where $F(\lambda)$ is the fluorescence intensity of fluorescence donor at wavelength of and $\varepsilon(\lambda)$ is molar absorbance coefficient of the acceptor when wavelength is λ.

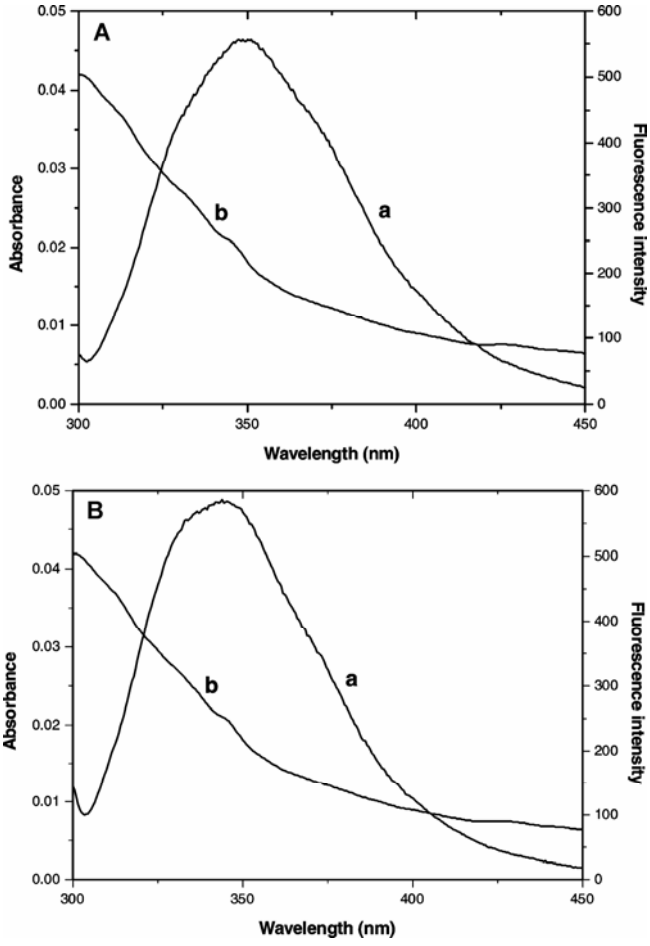

Figure 6. Overlapping between the fluorescence emission spectrum of α-Chy (a) (λex = 280 nm) and UV absorption spectrum of CdTe QDs (b). (**a**) pH = 9·05, (**b**) pH = 7·20; [α-Chy] = 1·6 × 10^{-6} M, [CdTe] = 3·6 × 10^{-5} M, T = 298 K.

In the present case, it turns out that $k^2 = 2/3$, $N = 1{\cdot}366$, and $\Phi = 0{\cdot}118$.[33] It was then calculated that $J = 2{\cdot}498 \times 10^{-15}$ cm^3 L mol^{-1}, $E = 0{\cdot}3051$, $r = 2{\cdot}232$ nm (pH = 9·05) and $E = 0{\cdot}4121$, $r = 2{\cdot}065$ nm (pH = 7·20). As the distance of donor to acceptor for CdTe–α-Chy binding is less than 8 nm, the energy transfer from α-Chy to CdTe occurs with high probability. From figure 7 it can be seen that the fluorescence intensity of QDs in presence of α-Chy is significantly higher than that of pure QDs and an iso-emission point occurs at 460 nm, which indicated that there are effective energy transfer between α-Chy and QDs, and the complex compound has been formed between α-Chy and QDs.

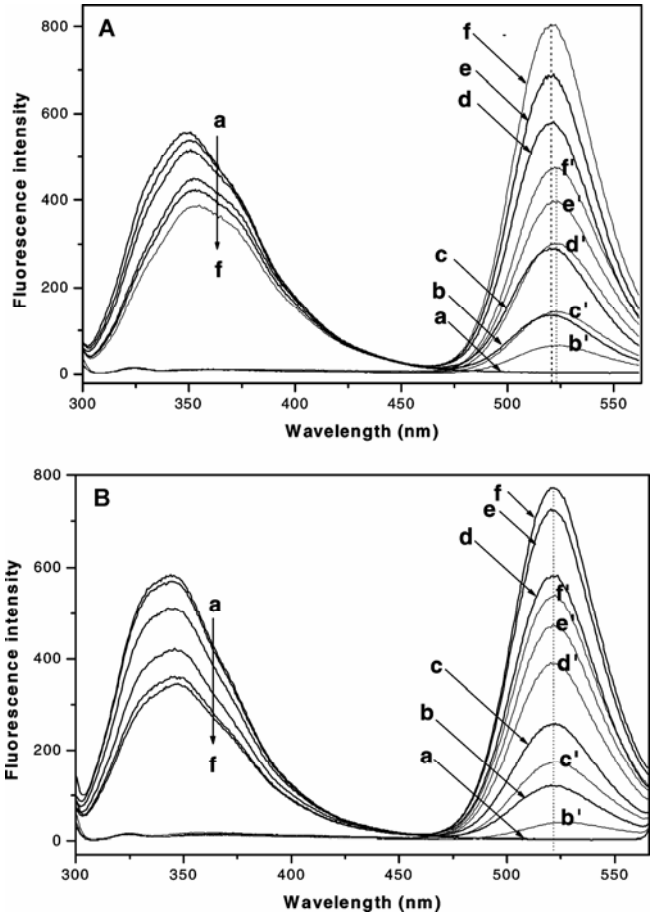

Figure 7. Energy transfer between α-Chy and CdTe QDs. (**A**) pH = 9·05 (**B**) pH = 7·20; The concentration of α-Chy from (a) to (f) was fixed as 1·6 × 10^{-6} M, the concentrations of CdTe QDs were 0, 6·00 × 10^{-6}, 1·20 × 10^{-5}, 2·40 × 10^{-5}, 3·00 × 10^{-5}, 3·60 × 10^{-5} M; the concentrations of CdTe QDs in the absence of α-Chy from (b′) to (f′) were 6·00 × 10^{-6}, 1·20 × 10^{-5}, 2·40 × 10^{-5}, 3·00 × 10^{-5}, and 3·60 × 10^{-5} M, respectively; T = 298 K.

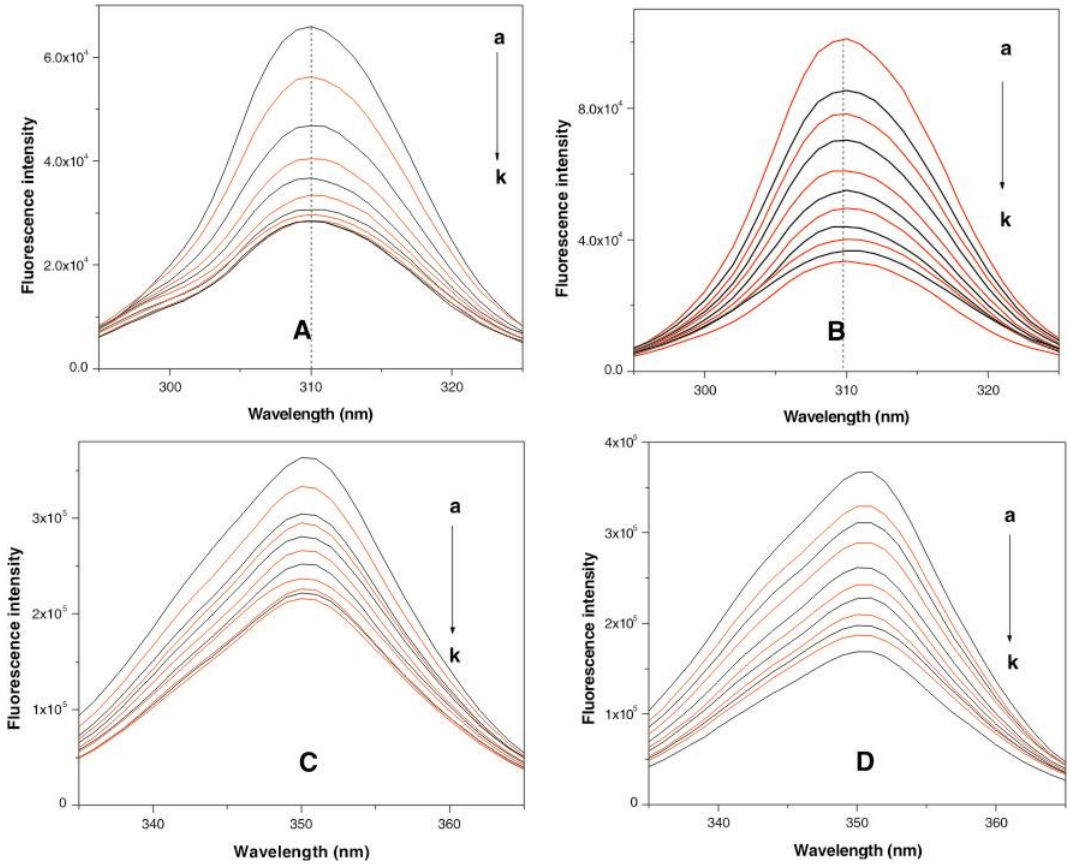

Figure 8. The synchronous fluorescence spectra of α-Chy with varying the concentration of CdTe QDs. (**A**, **B**) $\Delta\lambda$ = 15 nm, (**C**, **D**) $\Delta\lambda$ = 60 nm, (A, C) pH = 9·05, (**B**, **D**) pH = 7·20. The concentration of α-Chy was fixed as $1{\cdot}6 \times 10^{-6}$ M; the concentrations of CdTe QDs from (a) to (k) were 0, $6{\cdot}00 \times 10^{-6}$, $1{\cdot}20 \times 10^{-5}$, $1{\cdot}80 \times 10^{-5}$, $2{\cdot}40 \times 10^{-5}$, $3{\cdot}00 \times 10^{-5}$, $3{\cdot}60 \times 10^{-5}$, $4{\cdot}20 \times 10^{-5}$, $4{\cdot}80 \times 10^{-5}$, $5{\cdot}40 \times 10^{-5}$ and $6{\cdot}00 \times 10^{-5}$ M, respectively.

Table 3. Stability of α-chymotrypsin ($C = 1{\cdot}6 \times 10^{-6}$ M) in different concentration of CdTe.

pH	C(CdTe) $\times 10^{-5}$ M	Enzyme activity (ATEE U/mg)	0·5 h	2·5 h	6·5 h	10·5 h	24 h
			Relative enzyme activity (%)				
9·05	0	4375	100	97·14	94·29	95·13	91·43
	1·2		91·43	94·29	91·43	85·71	77·14
	6·0		89·25	85·71	80·00	79·43	74·29
	12·0		77·14	77·14	74·29	77·14	71·43
7·20	0	4000	100	98·13	96·88	93·75	93·13
	1·2		90·63	93·75	87·50	90·63	87·50
	6·0		87·50	84·38	81·25	78·13	77·50
	12·0		75·00	78·13	79·38	75·00	68·75

3.4 *Conformational investigations*

Synchronous fluorescence spectroscopy (SFS) is a fluorescence spectrum obtained by a synchronous motion of an excitation and emission monochromator at the same rate, but with a constant difference of wavelength $\Delta\lambda$. SFS can provide simplified spectrum with sharp emission peaks. As $\Delta\lambda$ between excitation wavelength and emission wavelength is 15 nm, synchronous fluorescence offers characteristics of tyrosine residues, when $\Delta\lambda$ is 60 nm, it provides the characteristic information of tryptophan residues.

Synchronous fluorescence spectra of α-Chy upon addition of CdTe QDs gained at $\Delta\lambda = 15$ and 60 nm are shown in figure 8 under two pH values.

As shown in figure 8, fluorescence intensity of both tryptophan and tyrosine decreases and a slightly red shift at maximum emission is observed on addition of CdTe, which indicates that the conformation of protein is changed. It is likely that due to the hydrophobic amino acid structure surrounding tryptophan and tyrosine residues in α-Chy tends to collapse slightly and thus tryptophan and tyrosine residues are more exposed to the aqueous phase.

CD is also a sensitive technique to monitor the conformational changes in proteins arising from interaction with a ligand. According to a quantitative analysis by Fourier transform infrared spectroscopy, α-Chy adopts a 50% β-structure in D_2O. α-Chy is a type of all-β proteins characterized by a CD spectrum which resembles that of a random coil conformation.[34] Figure 9 displays the CD spectra of α-Chy with various amounts of CdTe QDs under two pH values. It is noticed from figure 9 that the secondary structure of α-Chy is slightly different at two pH values. The CD spectrum of the native structure shows a global minimum around 205 nm, which has been attributed to the β-strand conformation in β-barrel architecture observed in the crystal structure of α-chymotrypsin (e.g. in 1ACB.pdb). Upon addition of CdTe QDs, the far-UV CD spectrum of the protein reveals a significant increase in signal intensities of 228 nm and decrease in the intensity of 203 nm, which indicated the α-helix and β-sheet structure content of α-Chy has been changed and it may be the result of the formation of complex between the α-Chy and CdTe.

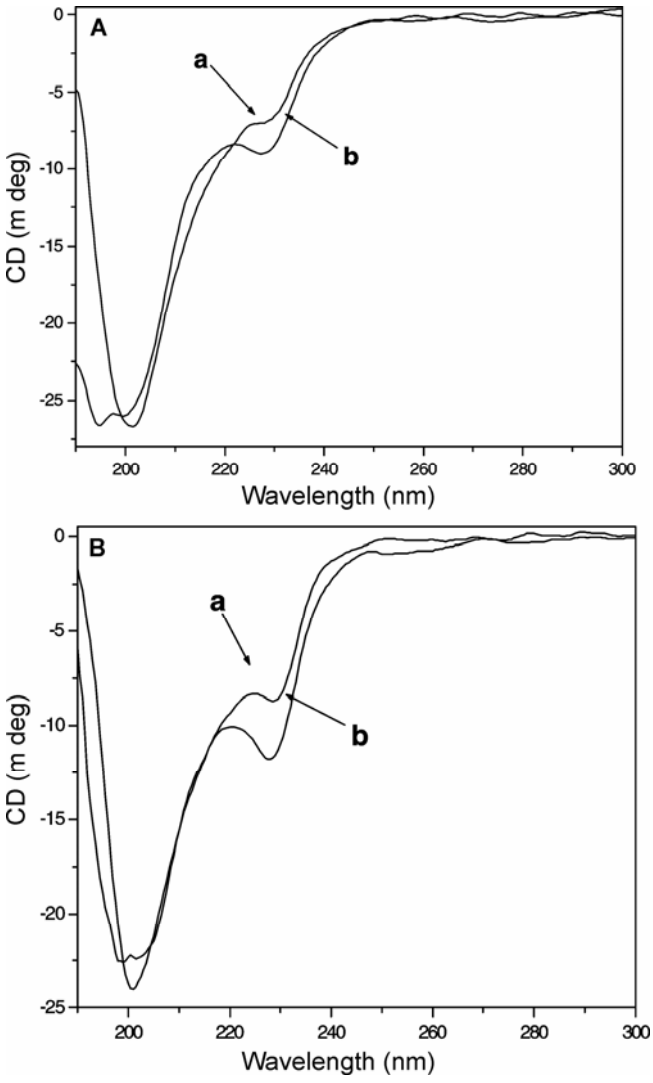

Figure 9. CD Spectra of the α-Chy -CdTe System. (**A**) pH = 9·05 (**B**) pH = 7·20; (a) $1{\cdot}6 \times 10^{-6}$ M α-Chy, (b) $1{\cdot}6 \times 10^{-6}$ M α-Chy in the presence of $6{\cdot}0 \times 10^{-5}$ M CdTe QDs.

3.5 *Assay of enzyme activities*

In order to investigate the activity and stability of α-Chy in the absence and presence of CdTe QDs, activities of α-Chy after storage in the two pH values (pH 9·05, pH 7·20) at 298 K were determined by measuring the absorbance at 237 nm. The measurements were performed at intervals of a few hours during a period of 24 h. As shown in table 3, when α-Chy in the absence of CdTe QDs show high activity. The activity of α-Chy still had a residual activity of 91·43% at pH 9·05 and 93·13% at pH 7·20. It should be noted that the high catalytic activities of the enzymes under two pH values can be explained not only by the similar overall structures of the proteins, but also by very little changes at the substrate binding site. It can be seen that the activity of α-Chy in the presence of a small quantity of CdTe had no significant loss. The results showed that α-Chy could maintain its high activity and stability in different pH values for 24 h.

4. Conclusions

The interactions between CdTe QDs and α-Chy were investigated by fluorescence quenching method. Results showed that CdTe quenched the fluorescence of α-Chy through static quenching mechanism. Hydrogen bonds or van der Waals force is involved in the binding process when pH is 9·05,

while the hydrophobic and electrostatic interactions are involved in the binding process when pH is 7·20. The distance (r) between donor and acceptor is obtained from Förster non-radioactive resonance energy transfer theory. Synchronous spectra and CD spectra reveal that CdTe QDs can change the conformation of α-Chy. Furthermore, α-Chy could maintain its high activity and stability under different pH values for 24 h in the presence of CdTe QDs.

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