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Modulation of Ligand Binding Affinity of Tumorigenic Carbonic Anhydrase XII upon Interaction with Cationic CdTe Quantum Dots†

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Abstract

The structural data of tumorigenic carbonic anhydrase (CA) XII revealed that the enzyme surface opposite to the active site pocket was negatively charged, and thus it had potential to interact with the positively charged surfaces. We investigated the influence of cationic CdTe quantum dots on the catalytic and ligand binding properties of the enzyme. Although cationic quantum dots interacted with CAXII (with a K_d value of 2.1 μM), they did not impair the enzyme's catalytic activity, suggesting that the accessibility of the enzyme's active site remained unaffected by the above interaction. When CAXII bound dansylamide (serving as a fluorescence probe as well as a potent inhibitor of the enzyme) was titrated with cationic quantum dots, the fluorescence spectral profiles revealed a marked transfer of the excited state energy between the above species. However, the binding of quantum dots to CAXII weakened the affinity of dansylamide for the enzyme, and thus obviated the inhibitory feature of the ligand. Since the quantum dots and dansylamide are bound at the opposite sides of CAXII, their influence must be mediated via changes in the protein conformation at a distal region as observed with allosteric enzymes. The mechanistic insights gained from these studies may lead toward developing diagnostic protocols for tumorigenic CAXII.

Keywords

Carbonic Anhydrase; CdTe Quantum dots; Fluorescence spectroscopy; Energy transfer; Tumor

1. INTRODUCTION

The notion that tumor cells are the selfish lineage of clones, which conform to the Darwinian principle of natural selection, is best exemplified by the multitude of survival strategies adopted by these cells under hypoxic conditions.¹ The rapidly proliferating tumor cells quickly overgrow such that the diffusion of oxygen from the nearest blood vessel (100–150 μm) is impaired.² Consequently, tumor cells receive low level of oxygen, causing local hypoxic centers and tissue necrosis. Hypoxia creates selective pressure in cells to adopt to stress conditions, resulting in the expression of approximately 50 additional proteins,³ including the enzymes involved in pH homeostasis. The latter is accomplished, at least in part, by an intricate coordination between selected carbonic anhydrase (CA) isozymes, particularly CA IX and CAXII.

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In the physiological milieu, there are 15 different isoforms of carbonic anhydrases, of which CAXII is localized on the surface of cell membranes. This enzyme along with its membrane associated partner, CA IX, are highly expressed in a variety of tumors, particularly under hypoxic conditions.^{4–6} The X-ray crystallographic structure of human CAXII⁷ reveals that it is a bitopic dimeric protein whose short intracellular C-terminus is placed in on the opposite side of the active site domains such that the latter face toward the extracellular space. A casual perusal of the electrostatic potentials on the surface of CAXII revealed that the region opposite to the enzyme's active site pocket was predominantly negatively charged (see Results). Hence, CAXII had potential to interact with the positively charged macro- or nano-clusters with its active site facing toward the aqueous environment thus exhibiting the potential to catalyze the reaction. With such an organizational feature in mind, we became interested in investigating the plausible functional consequences of interaction of CAXII with positively charged quantum dots. This is particularly so since the quantum dots exhibit distinct fluorescence spectral features,^{8,9} which could be tapped into probing the structural-functional aspects of the enzyme-ligand interactions. As will be shown in the subsequent sections, the use of CdTe quantum dots has unraveled the fact that its binding to CAXII weakens the affinity of an active site bound inhibitor (dansylamide) presumably via changes in the protein conformation.

2. MATERIALS AND METHODS

2.1 Materials

The plasmid encoding the catalytic domain region of the human carbonic anhydrase XII gene was a generous gift from Dr. William Sly's laboratory at St. Louis University School of Medicine (St. Louis, MO). Dansylamide was purchased from Avocado Research Chemicals (Heysham, Lanc., UK). The primers used for the PCR reactions were synthesized by Integrated DNA Technology (Coralville, IA). Cloned *Pfu* DNA polymerase and the BL21-CodonPlus[®] DE3 (RIL) *E. coli* cells were purchased from Stratagene (La Jolla, CA). Other restriction enzymes and T4 DNA ligase were obtained from New England Biolabs (Ipswich, MA). All other reagents were of analytical grade.

2.2 Preparation of CdTe Quantum Dots

The recipe for the synthesis of CdTe quantum dots is similar to what reported in literature.^{10–12} Briefly, CdTe nanoparticles were prepared by the reaction of precursors containing cadmium perchlorate hydrate [$\text{Cd}(\text{ClO}_4)_2 \cdot \text{H}_2\text{O}$] and hydrogen telluride (H_2Te) under vigorous stirring. The Cd^{2+} containing solution was prepared as follows: 0.7311 g of $\text{Cd}(\text{ClO}_4)_2 \cdot \text{H}_2\text{O}$ was dissolved in 125 mL of water. 0.396 ml of cysteamine was added to the solution and the pH was adjusted to ~11 by the addition of 0.1M NaOH. The solution was then purged with nitrogen for at least 30 minutes. H_2Te gas was generated by the chemical reaction of excess aluminum telluride with 0.5 M sulfuric acid in an inert atmosphere (nitrogen) and was combined with the above solution as described previously. After the completion of the reaction a yellowish solution of CdTe nanocrystal nuclei was obtained. This solution was then refluxed at 100 °C to promote crystal growth. The nanoparticles of different sizes were extracted at different reaction times and stored at 4 °C in the dark.

2.3 Cloning, Expression and Purification of the Recombinant Human CAXII

The sequence encoding human CAXII was amplified from the plasmid, pBShCAXII, obtained from Dr. William Sly's laboratory by the hot-start PCR method¹³ using the sense and anti-sense primers, 5' - GGAATTCCATATGTCCAAGTGGACT -TATTTTGGTCC - 3' and 5' - CGGGATCCTCATCAGAAGGAGGTGTATACCA - 3', respectively. The sense primer contained the *Nde I* cleavage site (CATATG) and the antisense primer contained the *BamH I* cleavage site (GGATCC). The PCR reaction mixture contained the template DNA (~0.1 µg), primers (5 µL of 25 µM), dNTPs (0.5 µL of 100 mM), MgCl_2 (10 µL of 25 mM), and *Pfu* DNA

polymerase (1 μ L of 2.5 units) in a total volume of 50 μ L. The PCR reaction conditions were set as follows: 1 min at 95 °C for denaturation, 1 min at 55 °C for annealing, 2 min at 72 °C for extension. This was repeated for a total number of 30 reaction cycles. An additional extension time of 20 min were allowed for the final products. *Nde I* and *BamH I* was used to digest the PCR reaction product and the pET-20b (+) plasmid expression vector. After digestion, both the product and vector were purified from an agarose gel using the Qiaquick[®] gel extraction kit (Qiagen; Valencia, CA). The purified products were ligated by T4 DNA ligase to form pET-hCAXII expression vector which was subsequently transformed into *E. coli* DH5 α cells by standard molecular biology protocol for plasmid propagation.¹³ The cloning of the coding regions of human CAXII in the pET-20b (+) vector was confirmed by sequencing of the plasmid at the University of Chicago Cancer Research Center (Chicago, IL).

The resulting pET-hCAXII plasmid was transformed into *Escherichia coli* BL21-CodonPlus[®] DE3 (RIL) cells by following the standard molecular biology protocol.¹³ After transformation, the cells were grown at 37 °C in LB medium, containing 100 μ g/mL of ampicillin, 50 μ g/mL of chloramphenicol, and 100 μ M of ZnSO₄ until A600 was 0.8. At this stage, the cells were allowed to grow at 18 °C until the absorbance reached 1.0 and were induced using 0.5 mM IPTG with the simultaneous addition of 200 μ M ZnSO₄. After induction, the cells were incubated at 18 °C for 12–16 hours with constant shaking in an orbital shaker at 300 rpm. The cell pellet was prepared by centrifugation at 5000 rpm for 15 min and the enzyme was purified as described by Banerjee *et al.* with only minor modifications.¹⁴

2.4 Calculation of the Electrostatic Surface Potential of CAXII

The X-ray crystallographic coordinates of human carbonic anhydrase XII were obtained from the Protein Data Bank (PDB ID: 1JCZ).⁷ The electrostatic surface potential of CAXII was calculated using the GRASP software program¹⁵ on an SGI molecular modeling workstation.

2.5 Measurements of CAXII Enzymatic Activity

The measurement of the activity of recombinant human CAXII was via the pH drop method of Wilbur and Anderson.¹⁶ In this method, the time taken for the decrease in pH of 0.02 M Tris-HCl buffer from 8.3 to 6.3 at 0–4 °C upon addition of a saturated CO₂ solution in the absence and presence of CA is determined. This procedure involves bubbling of CO₂ gas into ice cold water for about 30 minutes prior to the assay. During this process, the carbonated water is kept at 0–4 °C in an ice bath. In the blank reaction, 6.0 mL of chilled 0.02 M Tris-HCl, pH 8.3 is mixed with 4.0 mL of ice cold CO₂ saturated solution with constant stirring and the time required for the pH to drop from 8.3 to 6.3 is recorded. The above experiment is repeated in the presence of 0.25 μ M CAXII and the activity of the enzyme is determined by the following equation:

$$\text{Unit of CAXII Activity} = \frac{2 \times (T_0 - T)}{T} \quad \text{Eq. 1}$$

where T_0 and T are the time required to achieve the above pH drop in the absence and presence of the enzyme respectively.

2.6 Spectrofluorometric measurements

All the steady-state spectrofluorometric studies were performed on a Perkin Elmer LS 50B spectrofluorometer, equipped with a magnetic stirrer and thermostated water bath. As the CdTe quantum dots (QDs), prepared in Dr. Chen's laboratory, were found to be stable in 10 mM Tris, pH 8.0, all the experiments involving CdTe QDs were performed utilizing this buffer. A stock of 1 mM dansylamide was prepared in 10 mM HCl and was diluted into the above buffer

during the titration. The emission spectrum of the QDs was taken by fixing the excitation wavelength at either 440 nm or 570 nm.

The dissociation constant of CAXII-CdTe complex was determined by titrating a fixed concentration of CdTe by the enzyme in 10 mM Tris, pH 8.0. The excitation wavelength was set at 280 nm and the quenching of the protein fluorescence was monitored at 336 nm. A fixed concentration of quantum dots (0.23 μ M) was titrated with known concentrations of CAXII. A blank titration of protein into buffer was subsequently performed and the signal of the blank was subtracted from that of the sample. The difference in the protein emission intensity at 336 nm was plotted as a function of protein concentration and the dissociation constant of the CAXII-CdTe complex was determined as described by Qin and Srivastava.¹⁷

To determine the concentration of CdTe QDs, the fitting parameter corresponding to the QDs concentration was allowed to vary without constraints. The binding isotherm for the interaction of Dansylamide (DNSA) with CAXII was established in 25 mM HEPES, pH 7.0 containing 10% acetonitrile. The excitation and emission wavelengths were 330 nm and 457 nm, respectively, with excitation and emission slit widths of 5 nm. The dissociation constant for the CAXII-DNSA complex was determined via analysis of the data as described above.

3. RESULTS

To probe the possible electrostatic interactions of the recombinant form of human CAXII with charged surfaces, we determined the electrostatic surface potential of the enzyme using the X-ray crystallographic structural coordinates of Whittington *et al.* (PDB ID: 1JCZ).⁷ As depicted in Figure 1, the electrostatic surface potential of CAXII revealed that the surface of the enzyme is largely negatively charged, except for in the vicinity of active site which is positively charged. This finding suggested that CAXII had the potential to electrostatically interact with both positively and negatively charged surfaces with different orientations of the enzyme. Moreover, it appeared likely that the enzyme's active site region would interact with the negatively charged surfaces, whereas the opposite side of the enzyme would interact with the positively charged surfaces. Although the former orientation would likely inhibit the enzyme by occlusion of the active site pocket, the latter orientation would allow CAXII to remain active while bound to the positively charged surface.

To ascertain whether CAXII could bind to a positively charged surface, the binding of the enzyme to cationic quantum dots of approximately 4.5 nm diameter was investigated. The positive charge on the cadmium telluride (CdTe) quantum dots was prepared by the conjugation of cystamine. Figure 2 shows the absorbance and fluorescence spectra of the CdTe quantum dots. The cationic quantum dots (QD) show a broad absorption band from 250 nm to approximately 600 nm. Aside from the major peak in the UV region, a smaller peak was observed at 570 nm (Fig. 2, panel A). The latter peak was chosen for excitation of the QDs so as to avoid complications due to the absorbance of CAXII in the ultraviolet region. When excited at 570 nm, the steady-state fluorescence emission of the CdTe QDs was strong with an emission maximum of 610 nm (Fig. 2, panel B). However, despite the fluorescence signal provided by the quantum dots, no change in fluorescence was noted in the presence of CAXII. This finding seemed to suggest that CAXII was not binding to CdTe, regardless of the favorable electrostatic surface potentials between the charged species. However, such observation was also possible if the interacting species did not alter the electronic transition of the quantum dots despite their physical interactions.

To probe whether the intrinsic fluorescence of CAXII was influenced by the cationic QDs, we determined fluorescence spectra of the enzyme in the presence of increasing concentrations of QDs. The catalytic domain of CAXII harbors four tryptophan residues that, upon excitation at

280 nm, exhibit an emission maximum at 336 nm. As the steady-state fluorescence emission of tryptophan is highly sensitive to changes in its microenvironment,¹⁸ it appeared plausible that this signal would change upon binding of CAXII to the positively charged QDs. It was found that the steady-state fluorescence of the protein was indeed quenched in the presence of CdTe QDs. The latter served as a signal for monitoring the binding of CAXII to QDs. However, prior to determining the binding affinity of CAXII for QDs, it was realized that the titration of an increasing concentration of the latter to a fixed concentration of protein was not feasible. This was a consequence of the significant UV absorption of the QDs (as evident by the spectra in panel A of Fig. 2). Since increasing concentrations of CdTe QDs would produce inner filter effect, it would prevent the determination of a reliable binding constant by artifactually lowering the tryptophan fluorescence emission. Hence, to determine the binding affinity of CAXII for cationic QDs, a titration of a fixed concentration of QDs with enzyme was performed while monitoring the fluorescence emission of CAXII at 336 nm. The observed fluorescence was corrected by subtraction of a background titration of CAXII into buffer and then plotted as a function of enzyme concentration (Figure 3).

As is evident from the data of Fig. 3, the fluorescence of CAXII exhibited a saturating (hyperbolic) behavior in the presence of QDs. This finding was consistent with the formation of a complex between CAXII and CdTe QDs. For the analysis of the binding isotherm, it should be emphasized that although the concentration of CAXII was known, we had no method to directly determine the concentration of CdTe QDs. Hence, we could not ascertain *a priori* whether it was valid to assume that the total concentration of CAXII was approximately equal to the free concentration of the enzyme throughout the titration. Therefore, a binding equation accounted for both the concentrations of the enzyme and the QDs had to be employed¹⁷ to determine the dissociation constant (K_d). The solid smooth line in Fig. 3 represents the best fit of the data to the quadratic binding model as described by Qin and Srivastava¹⁷ which provided a dissociation constant (K_d) of 2.1 μM for CAXII-CdTe complex. The fitting results yielded the concentration of QDs as being 0.23 μM . The concentration of CdTe QDs calculated from this analysis was then utilized to determine the concentration of the QDs stock for further use.

Since the data of Fig. 3 indicated that CAXII was able to bind to cationic QDs, it was of interest to determine whether the binding of CAXII to CdTe active site structure of the enzyme. Toward this goal, we compared the binding affinity of CAXII for the inhibitor, dansylamide, in the absence and presence of QDs. Dansylamide (DNSA) is a highly fluorescent competitive inhibitor of carbonic anhydrases which undergoes a blue shift in the fluorescence emission spectrum concomitant with the increase in the quantum yield of the enzyme's active site resident fluorophore.^{19–21} It was reasoned that if a change in the active site structure of CAXII were to occur upon binding to the quantum dots, the binding of DNSA to the CAXII-CdTe complex would be altered. To probe this, we first determined the binding affinity of CAXII for DNSA by titration of DNSA into CAXII while monitoring the increase in fluorescence intensity of the CAXII-DNSA complex. The resulting binding isotherm for the formation of CAXII-DNSA complex is depicted in Figure 4. The solid smooth line is the best fit of the data to the quadratic binding equation,¹⁷ which provided a K_d of 80 nM. This K_d obtained for CAXII-DNSA is much lower than that exhibited by other carbonic anhydrase isozymes which bind to DNSA.¹⁴

Having established the binding affinity of free CAXII for DNSA, the binding of DNSA by CAXII in the presence of CdTe QDs was investigated. A fixed concentration of CAXII (1 μM) was titrated with increasing concentrations of the cationic QDs in the presence of a saturating concentration of DNSA (5 μM) and the fluorescence of CAXII-DNSA complex was monitored. The changes in steady-state fluorescence emission intensity as a function of the concentration of QDs are shown in Figure 5. As the concentration of QDs were increased, a decrease in the fluorescence intensity of the CAXII-DNSA emission peak at 457 nm was

observed. Furthermore, after subtraction of the background signal of free CdTe QDs, an increase in the fluorescence emission intensity of the QDs was evident (with a slight blue shift in the emission maxima from 610 to 600 nm) as a function of the concentration of QDs.

The decrease in the 457 nm peak apparent in Fig. 5 suggested that DNSA was excluded from the CAXII active site pocket in the presence of CdTe QDs. The removal of DNSA from enzyme in the presence of the latter was attributed to either the decrease in the binding affinity of CAXII for DNSA in the presence of quantum dots or the direct displacement of the ligand upon binding to QDs. Hence, we attempted to determine the binding affinity of CAXII for DNSA in the presence of CdTe QDs by titration of DNSA into CAXII in the presence of a saturating concentration of quantum dots, while monitoring the signal of the enzyme bound DNSA. However, no signal was observed for the CAXII-DNSA complex which indicated that CAXII could not bind DNSA while already bound to QDs or that the fluorescence of CAXII-bound DNSA was quenched in the presence of the QDs.

The increase in the fluorescence intensity of CdTe QDs depicted in Fig. 5 was in contrast with the above observation that formation of CAXII-CdTe complex did not result in a change in the emission of the QDs. To ascertain whether this discrepancy was from the direct interaction of the free DNSA with the CdTe, the fluorescence emission spectrum of the latter in the presence of DNSA was determined (Figure 6). When compared to the fluorescence spectra of the QDs alone as well as that of CAXII-CdTe complex, it was clear that the fluorescence emission of the QDs was slightly increased in the presence of DNSA. This increase suggested an apparent interaction between CdTe QDs and DNSA, albeit to a minor extent compared to the interaction of QDs with CAXII-DNSA complex. Thus, it was surmised that the increase in fluorescence emission intensity of the QDs observed in Fig. 5 was mainly a consequence of the interaction with CAXII-bound DNSA and not due to the interaction of the QDs with free enzyme or inhibitor. However, since binding of CAXII to the QDs did not perturb the electronic environment of the latter, it was conjectured that the increase in the intensity of the QDs fluorescence in the presence of CAXII-DNSA complex was a consequence of fluorescence resonance energy transfer (FRET) from the CAXII-bound DNSA to the QDs. The excited state energy transfer from DNSA to QDs was consistent with the concomitant decrease in the emission intensity of the CAXII-bound DNSA upon titration with QDs (Fig. 5). This suggested that the decrease in the fluorescence intensity of the CAXII-bound DNSA in the presence of QDs was a consequence of not only the decreased binding affinity of DNSA for the CAXII-QD complex but also the FRET from the CAXII-bound inhibitor to the QDs.

Since the binding of DNSA to CAXII results in the inhibition of the enzyme, we explored the effect of QDs on the DNSA-inhibited enzyme activity. The activity of a fixed concentration of CAXII (0.25 μ M) was measured in the absence and presence of QDs and/or DNSA using the pH drop assay.¹⁶ In the absence of QDs and DNSA, the enzyme activity was found to be 20 units. This activity remained unchanged upon addition of a saturating concentration of quantum dots, indicating that the formation of CAXII-CdTe complex did not inhibit the activity of the enzyme. In contrast, the treatment of CAXII with DNSA resulted in the decrease in the activity of CAXII by about 90% of the activity of the free enzyme. However, upon treatment of the CAXII-DNSA complex with QDs, it was observed that the full activity (20 units) of the enzyme was regained. These results demonstrated that although the binding of CAXII to cationic QDs did not affect the catalytic properties of the enzyme, the QDs could reverse the inhibitory effect of the enzyme by DNSA. A molecular mechanism of this unprecedented observation is elaborated in the next section.

4. DISCUSSION

A comprehensive account of the experimental data described in the earlier section for the interaction of CAXII with CdTe QDs has lead to several conclusions. (1) CAXII is able to bind to positively charged QDs through electrostatic interactions to form CAXII-CdTe complex. (2) The binding of CAXII to CdTe QDs perturbed the intrinsic fluorescence emission of CAXII without altering the fluorescence emission of the QDs. (3) DNSA bound to CAXII transfers the excited state energy to CdTe QDs within the CAXII-CdTe complex (4) The binding of QDs to CAXII-DNSA complex decreases the binding affinity of enzyme for DNSA. (5) CAXII remains fully active upon binding to QDs and the activity of CAXII-CdTe complex is not inhibited by DNSA.

The fact that the intrinsic tryptophan fluorescence of CAXII was quenched upon binding of the enzyme to the QDs suggested their plausible interaction. This was not surprising in the light of the oppositely charged surfaces between CAXII and the QDs, which could promote electrostatically-mediated interactions between the above species. It can be surmised that upon binding of CAXII to cationic QDs, the active site pocket of the enzyme would likely be oriented away from the surface of the latter. This is because the positive charges of the QDs would interact with the negative charges of the opposite face of the enzyme. Support for this orientation of CAXII is provided by the observation that the binding of the enzyme to the CdTe QDs does not affect the activity of the enzyme. This would not be the case if the enzyme was oriented such that the active site pocket was oriented towards the QD surface as this would occlude the accessibility of the substrate during catalysis.

The conclusion that the orientation of the QD-bound CAXII is such that the active site pocket of the enzyme is distant from the surface of the QDs is valid despite the fact that the binding of CAXII to DNSA is weakened in the presence of QDs. The latter finding was evidenced by the lack of an isosbestic point in the spectral data of Fig. 5. Since the fluorescence emission of free DNSA exhibits a maximum at 536 nm, the increase in the fluorescence band at the above wavelength was responsible for precluding clean isosbestic points upon titration of CAXII-DNSA complex by the QDs (Fig. 5). If not for the activity of the CAXII-CdTe complex, it would be tempting to argue that the loss of DNSA from the enzyme phase was due to the direct displacement of the inhibitor upon binding of QDs to the CAXII-DNSA complex. However, as the direct displacement of DNSA from the enzyme phase by QDs would likely be due to the occlusion of the active site, this was deemed to be unlikely. Instead it appeared that the presence of QDs decreased the binding affinity DNSA for CAXII via changes in the protein conformation. This observation is similar to those observed in the case of allosteric enzymes and thus it is tempting to speculate that cationic QDs serve as allosteric effectors in modulating the avidity of the CAXII active site for inhibitors.

Although the binding affinity of CAXII for DNSA decreases, it appears that not all of the DNSA is displaced from the enzyme site upon binding with QDs as evident from the fact that the fluorescence intensity of the QDs increases in the presence of the CAXII-DNSA complex (Fig. 5). Since the binding of CAXII to QDs does not increase the fluorescence emission intensity of the latter, it is likely that FRET from enzyme-bound DNSA to the QDs occurs and is responsible for the increase in the fluorescence intensity of the latter. Such an energy transfer would explain both the decrease in the signal of the enzyme-bound DNSA and the lack of this signal upon titration of DNSA into CAXII-CdTe complex. By this reasoning, it is likely that at least some DNSA was still bound to CAXII in the presence of QDs, but was rendered spectroscopically silent due to the FRET. We are currently in the process of investigating the microscopic rationale for the observed changes in fluorescence and will report our findings subsequently.

A cumulative account of our experimental outcomes has led to depiction of a tentative model for the interaction of CAXII with QDs in the absence and presence of DNSA (Figure 7). The model of Fig. 7 shows the binding of CAXII to QDs takes place at the side opposite to the active site pocket of the enzyme. Due to the rigid surface of the QDs as compared to the relatively flexible CAXII, upon binding the enzyme is proposed to “flatten out” (via conformational changes) on the surface of the QDs. The conformational changes would not be restricted to the interacting face of the enzyme and would result in the opening up of the active site pocket. However, despite the structural changes in the active site, the catalytic properties of CAXII are not affected when bound to CdTe QDs. This is because the substrates of the enzyme (CO_2 and H_2O) are very small molecules and they make only a few direct contacts with the enzymes active site, such as the formation of the Zn^{2+} -bound hydroxide.^{22,23} On the other hand, the QD-induced changes in the enzyme conformation appear to be adequate to alter the binding affinity of DNSA to CAXII and cause the slight displacement of the inhibitor. This is because DNSA is much larger than CO_2 and H_2O and makes more extensive contacts with the enzyme. Hence, although the inhibitor can still remain bound to the CAXII-CdTe complex, its binding affinity can be impaired, precluding the ability of DNSA to effectively block the active site. This feature allows for catalysis to proceed as the entrance of CO_2 and H_2O to the active site and the release of the bicarbonate product are unobstructed. We are currently in the process of fine-tuning our model to discern the molecular basis of cationic QD-mediated allosteric phenomenon in CAXII. Such studies are expected to shed light on the role of CAXII in hypoxia-induced carcinogenesis.

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References

1. Harris AL. Hypoxia--a key regulatory factor in tumour growth. *Nat Rev Cancer* 2002;2:38–47. [PubMed: 11902584]
2. Richard DE, Berra E, Pouyssegur J. Angiogenesis: how a tumor adapts to hypoxia. *Biochem Biophys Res Commun* 1999;266:718–722. [PubMed: 10603309]
3. Potter C, Harris AL. Hypoxia inducible carbonic anhydrase IX, marker of tumour hypoxia, survival pathway and therapy target. *Cell Cycle* 2004;3:164–167. [PubMed: 14712082]
4. Wykoff CC, Beasley NJ, Watson PH, Turner KJ, Pastorek J, Sibtain A, Wilson GD, Turley H, Talks KL, Maxwell PH, Pugh CW, Ratcliffe PJ, Harris AL. Hypoxia-inducible expression of tumor-associated carbonic anhydrases. *Cancer Res* 2000;60:7075–7083. [PubMed: 11156414]
5. Ivanov SV, Kuzmin I, Wei MH, Pack S, Geil L, Johnson BE, Stanbridge EJ, Lerman MI. Down-regulation of transmembrane carbonic anhydrases in renal cell carcinoma cell lines by wild-type von Hippel-Lindau transgenes. *Proc Natl Acad Sci USA* 1998;95:12596–12601. [PubMed: 9770531]
6. Türeci O, Sahin U, Vollmar E, Siemer S, Göttert E, Seitz G, Parkkila AK, Shah GN, Grubb JH, Pfreundschuh M, Sly WS. Human carbonic anhydrase XII: cDNA cloning, expression, and chromosomal localization of a carbonic anhydrase gene that is overexpressed in some renal cell cancers. *Proc Natl Acad Sci USA* 1998;95:7608–7613. [PubMed: 9636197]
7. Whittington DA, Waheed A, Ulmasov B, Shah GN, Grubb JH, Sly WS, Christianson DW. Crystal structure of the dimeric extracellular domain of human carbonic anhydrase xii, a bitopic membrane protein overexpressed in certain cancer tumor cells. *Proc Natl Acad Sci USA* 2001;98:9545–9550. [PubMed: 11493685]
8. Chen W. Nanoparticle fluorescence based technology for biological applications. *J Nanosci Nanotechnol* 2008;8:1019–1051. [PubMed: 18468106]

9. Morgan NY, English S, Chen W, Chernomordik V, Russo A, Smith PD, Gandjbakhche A. Real time in vivo non-invasive optical imaging using near-infrared fluorescent quantum dots. *Acad Radiol* 2005;12:313–323. [PubMed: 15766692]
10. Joly AG, Chen W, McCready D, Malm J, Bovin J. Upconversion luminescence of CdTe nanoparticles. *Phys Rev B* 2005;71:165304.
11. Gaponik N, Talapin DV, Rogach AL, Hoppe K, Shevchenko EV, Kornowski A, Eychmuller A, Weller H. Thiol-capping of CdTe nanocrystals: an alternative to organometallic synthetic routes. *J Phys Chem B* 2002;106:7177–7185.
12. Qin Y, Yang X, Yu J. Synthesis and characterization of CdTe nanoparticles stabilized by cysteamine. *Chinese J Inorg Chem* 2006;22:851–855.
13. Sambrook, J.; Fritsch, EF.; Maniatis, T., editors. *Molecular cloning: A laboratory manual*. Vol. 1. Cold Spring Harbor Laboratory Press; Plainview, NY: 2000.
14. Banerjee AL, Tobwala S, Ganguly B, Mallik S, Srivastava DK. Molecular basis for the origin of differential spectral and binding profiles of dansylamide with human carbonic anhydrase I and II. *Biochemistry* 2005;44:3673–3682. [PubMed: 15751944]
15. Nicholls A, Sharp KA, Honig B. Protein folding and association: insights from the interfacial and thermodynamic properties of hydrocarbons. *Proteins* 1991;11:281–296. [PubMed: 1758883]
16. Wilbur KM, Anderson NG. Electrometric and colorimetric determination of carbonic anhydrase. *J Biol Chem* 1948;176:147–154. [PubMed: 18886152]
17. Qin L, Srivastava DK. Energetics of two-step binding of a chromophoric reaction product, trans-3-indoleacryloyl-coa, to medium-chain acyl-coenzyme-a dehydrogenase. *Biochemistry* 1998;37:3499–3508. [PubMed: 9521671]
18. Lakowicz, JR., editor. *Principles of fluorescence spectroscopy*. Vol. 1. Springer; Hoboken: 1999.
19. Chen RF, Kernohan JC. Combination of bovine carbonic anhydrase with a fluorescent sulfonamide. *J Biol Chem* 1967;242:5813–5823. [PubMed: 4990698]
20. Nair SK, Krebs JF, Christianson DW, Fierke CA. Structural basis of inhibitor affinity to variants of human carbonic anhydrase II. *Biochemistry* 1995;34:3981–3989. [PubMed: 7696263]
21. Day YSN, Baird CL, Rich RL, Myszkowski DG. Direct comparison of binding equilibrium, thermodynamic, and rate constants determined by surface-and solution-based biophysical methods. *Protein Sci* 2002;11:1017–1025. [PubMed: 11967359]
22. Liang JY, Lipscomb WN. Binding of substrate co2 to the active site of human carbonic anhydrase ii: a molecular dynamics study. *Proc Natl Acad Sci USA* 1990;87:3675–3679. [PubMed: 2111014]
23. Lindskog S. Structure and mechanism of carbonic anhydrase. *Pharmacol Ther* 1997;74:1–20. [PubMed: 9336012]

Figure 1.

Electrostatic surface potential of the catalytic domain of human carbonic anhydrase XII.⁷ The active site pocket of CAXII is oriented towards the upper left of the figure whereas the enzyme face that binds the QDs is in the lower right. The electrostatic potential of the enzyme surface was calculated using the GRASP software program¹⁵ on an SGI molecular modeling workstation. Regions of the negative and positive charges are color-coded red and blue, respectively.

Figure 2. Absorption and fluorescence emission spectra of the CdTe QDs (panels A and B, respectively). Fluorescence emission was monitored by excitation of the CdTe QDs at a wavelength of 430 nm.

Figure 3.

Binding isotherm for the interaction of CAXII with CdTe QDs. A fixed concentration of the quantum dots (0.23 μM) was titrated with increasing concentrations of CAXII in 10 mM Tris buffer, pH 8.0. The excitation was at 280 nm and the protein fluorescence was monitored at 336 nm. The data represent the corrected intensity after subtraction of a blank titration of CAXII into buffer. The solid smooth lines is the best fit of the data to a single binding site model¹⁷ providing a K_d of 2.1 μM.

Figure 4.

Binding isotherm for the interaction of dansylamide (DNSA) with CAXII. The experiments were performed in 25 mM HEPES, pH 7.0 containing 10% acetonitrile. The data represent the increase in the fluorescence emission intensity at 457 nm (excitation at 330 nm) for the titration of a fixed concentration of the enzyme (0.5 μM) with DNSA. The solid smooth line is the best fit of the data to a single binding site model¹⁷ with a K_d of 0.08 μM.

Figure 5.

Steady-state fluorescence emission spectra of CAXII DNSA complex as a function of increasing concentrations of CdTe QDs. Emission spectra were measured in 10 mM Tris buffer, pH 8.0 containing 10% acetonitrile with concentrations of CAXII and DNSA being equal to 1 and 5 μ M, respectively. Excitation of the samples was at 330 nm and fluorescence emission was monitored from 400–630 nm. The solid lines depict the corrected fluorescence emission spectra after subtraction of the spectra of free QDs. For clarity, arrows have been added to indicate the changes of each band upon titration with QDs.

Figure 6.

Steady-state fluorescence emission spectra of the CdTe QDs. The solid green line depicts the spectrum of free CdTe which overlays with the spectrum of CdTe in the presence of 1 μ M CAXII. The solid black line indicates the fluorescence emission spectrum of the QDs in the presence of 5 μ M DNSA. All spectra were measured in 10 mM Tris, pH 8.0 containing 10% acetonitrile with an excitation wavelength of 500 nm.

Figure 7.

Proposed model for the interaction of CA XII with cationic CdTe QDs in the absence and presence of DNSA. The figures represent the active and inhibited forms of CAXII (shown in cyan and pink, respectively), DNSA (green oval shape) and the QDs (yellow spheres) whereas the (+) and (-) symbols indicate the positive and negative charges, respectively, of the QDs and enzyme. The cartoon depicts the binding of CAXII to QDs which causes a conformational change in the active site pocket of the enzyme in the absence and presence of DNSA. The conformational change of CAXII upon binding to QDs maintains full activity of the enzyme, but decreases the binding affinity for DNSA. This latter feature prevents DNSA from completely blocking the active site pocket of the QD-bound CAXII and thus allows for catalysis to occur.