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Molecular Basis of Binding and Stability of Curcumin in Diamide-Linked γ-Cyclodextrin Dimers

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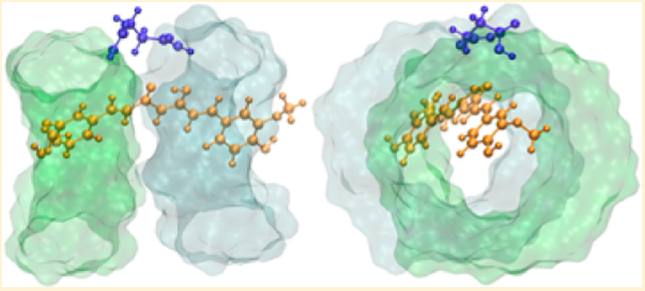
Supporting Information

ABSTRACT: Curcumin is a naturally occurring molecule with medicinal properties that is unstable in water, whose efficacy as a drug can potentially be enhanced by encapsulation inside a host molecule. In this work, the thermodynamics and mechanism of binding of curcumin to succinamide- and urea-linked γ-cyclodextrin (γ-CD) dimers in water are investigated by molecular dynamics simulations. The simulated binding constants of curcumin to succinamide- and urea-linked γ-CD dimers at 310 K are 11.3×10^6 M^{-1} and 1.6×10^6 M^{-1}, respectively, matching well with previous experimental results of 8.7×10^6 M^{-1} and 2.0×10^6 M^{-1}. The simulations reveal structural information about the encapsulation of curcumin inside the diamide-linked γ-CD dimers, with distinct qualitative differences observed for the two dimers. In particular, (1) the predominant orientation of curcumin inside the urea-linked γ-CD dimer is perpendicular to that in the succinamide-linked γ-CD dimer; (2) the magnitude of the angle between the planes of the cyclodextrins is larger for the succinamide-linked γ-CD dimer; and (3) curcumin exhibits greater configurational freedom inside the urea-linked γ-CD dimer. A consequence of some of these structural differences is that the dimer interior is more accessible to water in the succinamide-linked γ-CD dimer. These observations explain the higher stability and lower binding constant observed experimentally for curcumin in the urea-linked cyclodextrin γ-CD dimer compared with the succinamide-linked γ-CD dimer. More generally, the results demonstrate how stability and binding strength can be decoupled and thus separately optimized in host−guest systems used for drug delivery.



INTRODUCTION

Curcumin (Figure 1a) is a biomolecule of great interest due to its highly desirable anti-cancer,[1,2] anti-inflammatory,[1,3] anti-Alzheimer's,[4,5] wound healing,[6−10] and other medicinal properties.[11−13] However, two chemical obstacles must be overcome before curcumin's medicinal properties can be exploited. First, curcumin's low solubility in aqueous environments limits its bioavailability. Second, the stability of curcumin in the aqueous environment is poor: it is susceptible to hydrolysis at the keto−enol moiety, readily degrading in water within 30 min.[14,15] A number of encapsulation and delivery strategies have been investigated, including micelles,[16] polymer nanoparticles,[17,18] globular proteins,[14] and cyclodextrins.[19−23] One of the most promising of these strategies involves using diamide-linked γ-cyclodextrin dimers.[22] γ-Cyclodextrin (γ-CD) is a naturally occurring cyclic oligosaccharide consisting of 8 glucopyranoside units (Figure 1b). The structure of γ-CD, with its hydrophobic interior and hydrophilic exterior, presents a suitable environment for solubilizing and stabilizing curcumin. Work by Harada et al.[22] has demonstrated the potential of two such diamide-linked γ-CD dimers as encapsulation and delivery agents for curcumin. The two diamide linkers used, urea and succinamide (Figure 1c and 1d, respectively), both increased the stability of curcumin in water, by a factor of 780 and 180, respectively. The measured 1:1 binding constants at 310 K are 2.0×10^6 M^{-1} and 8.7×10^6 M^{-1} for urea and succinamide, respectively. These binding and stability results seem counterintuitive, as a higher binding constant is normally associated with an increased stability if the linked γ-CD dimer is protecting curcumin from hydrolysis. However, Figure 1c and d represent simple schematics for illustrating the encapsulation of curcumin, and so they are limited in their ability to explain the observed binding and stability. In order to rationalize this behavior, molecular dynamics simulations were used in this work to determine the behavior of the linked γ-CD dimers and of curcumin within them. Figure 2 shows a snapshot from one such simulation. Molecular dynamics simulations of curcumin in the past have focused on its interaction with DNA,[24] metalloenzymes,[25] beta-amyloid fibrils,[4] and a range of other targets. Similarly, simulations of cyclodextrins have focused on their role as host molecules to encapsulate smaller guest molecules[26−28] or on structural differences between α-, β-, and γ-CDs in water.[29] The binding energies of CDs with various guest molecules have been investigated in several studies.[30−32] The self-interaction of CDs in the formation of dimers or larger molecular structures has been examined in other studies.[33−35]

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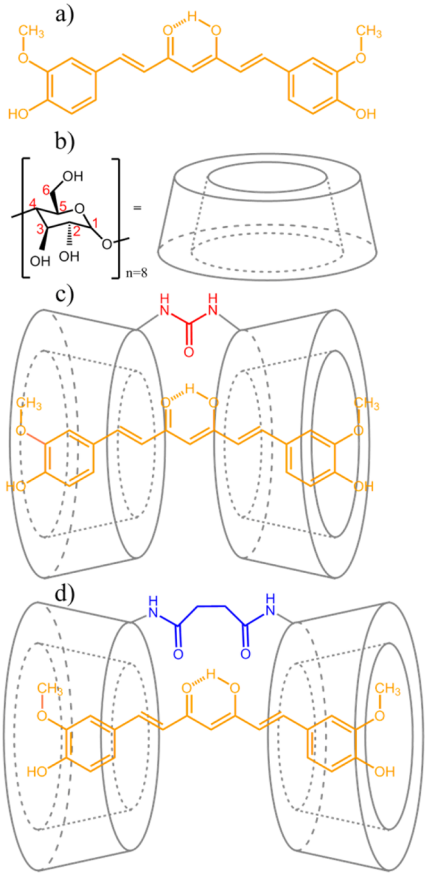

Figure 1. Structures of (a) curcumin, (b) γ-cyclodextrin (γ-CD), and curcumin in (c) urea-linked and (d) succinamide-linked γ-CD dimers.

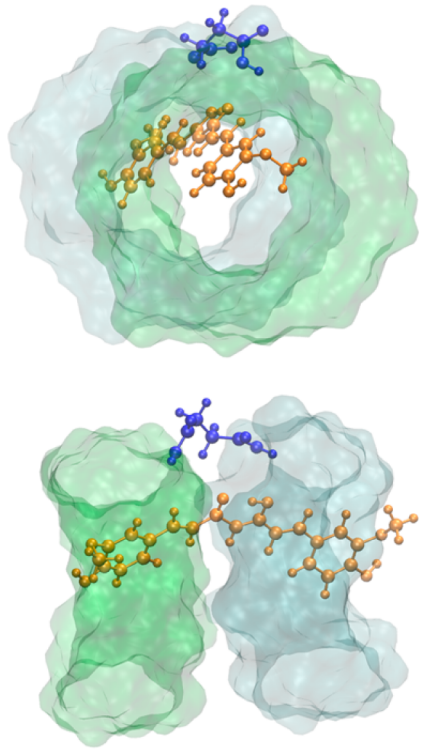

Figure 2. Simulation snapshot of curcumin in succinamide-linked γ-CD dimer from side (top) and front (bottom). Water is not shown for clarity.

This work is the first to simulate diamide-linked γ-CDs and their interactions with curcumin and elucidates the relationships between the properties of the diamide linker and the binding constant and stabilization of curcumin. It also demonstrates the possibility of designing host−guest systems in which stability and binding strength are decoupled and can thus be separately optimized. This is a potentially useful property for drug delivery, in which high drug stability is desirable, while excessively strong binding might limit efficient delivery.

COMPUTATIONAL METHODS

Force Field Details. The force field parameters for the γ-CD component of the γ-CD dimers were taken from the q4md-CD Amber-derived force field developed by Cézard et al.[36] The q4md-CD force field reproduces the structural, geometrical, and dynamic properties of CDs better than the GLYCAM04, GLYCAM06, or Amber99SB force fields. The diamide linkers, urea and succinamide, were parametrized using Antechamber, a program that aids in the parametrization of novel molecules based on Amber force fields. The General Amber Force Field (GAFF) was used for the bonded and nonbonded terms of the linkers.[37] The charges were determined by the restrained electrostatic potential (RESP) method.[38] An *ab initio* geometry optimization of the diamide linker connected to three glucopyranoside units on each side *in vacuo* was performed using HF/6-31G* in Gaussian 09 (B.02).[39] The optimized structure was used in Antechamber to generate a Gaussian input file to determine the charges on each atom using the RESP method. Three glucopyranoside units were used instead of the entire cyclodextrin unit to reduce the computational demands of the calculation. The bond and angle parameters for the C−N linkage between the cyclodextrin and diamide were taken from the GLYCAM04 force field. Curcumin was parametrized using GAFF and Antechamber following the same method as above. The GAFF force field is compatible with Amber-based force fields and can be used as an initial guess for refining more accurate force field parameters.[37] The TIP3P model, which is compatible with the GAFF[40] and q4md-CD[36] force fields, was used for the water molecules.

The GAFF and GLYCAM04 parameters used for the diamide linkers and the q4md-CD parameters for peptidic fragments such as diamide linkers are very similar: the equilibrium bond lengths and angles are within 0.01 Å and 2°, respectively, in all cases, and proper and improper dihedral potentials are identical. The bond and angle force constants differ more substantially, but the differences in the strengths of these short-range and fairly rigid bonding interactions are not expected to affect significantly the large-scale structural changes that the cyclodextrin dimers undergo in encapsulating curcumin.

Simulation Details. Molecular dynamics (MD) simulations were performed using NAMD 2.8.[41,42] Data analysis and processing used custom scripts and VMD.[43,44] Curcumin was placed in the diamide-linked γ-CD dimers in three orientations (at 0°, 90°, and 180° with respect to the linker − see Figure 3c). The *solvate* plugin for VMD was used to add water molecules to the system with a buffer distance of 10 Å. A custom script was used to set the number of water molecules to 3140 for all systems. The energy of each simulated system was minimized and then the system was gradually heated from 25 to 298 K over 55.2 ps. Simulations were then run for 50 ns in the NPT ensemble at a temperature of 298 K and pressure of 1 atm

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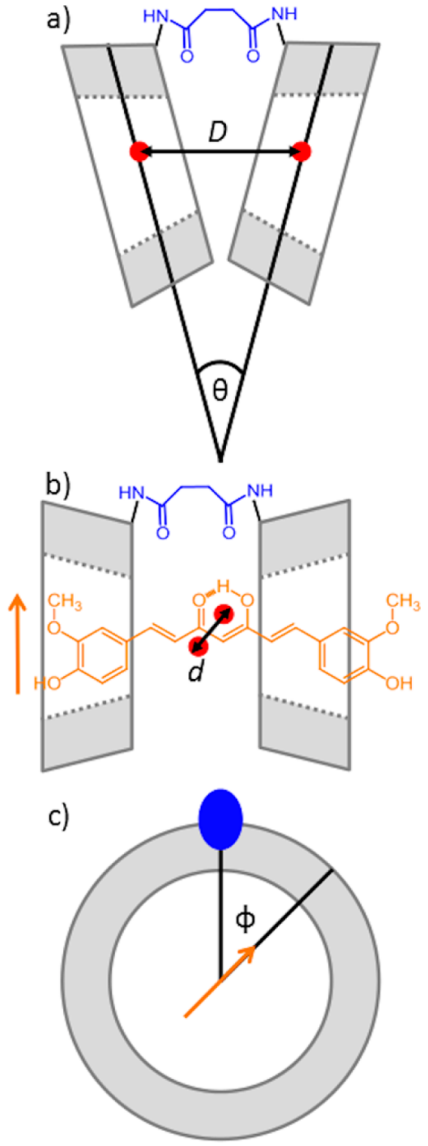

Figure 3. Schematic of measurements used: (a) angle θ between CD planes and distance D between geometric centers of cyclodextrins;. (b) distance d between geometric center of curcumin and the diamide-linked γ-CD dimer; (c) orientation angle ϕ of curcumin with respect to the diamide linker (the direction of the arrow defining curcumin's orientation is illustrated in (b)).

(101.3 kPa) using a Langevin thermostat and barostat. Analogous simulations of curcumin in the two diamide-linked γ-cyclodextrin dimers were also carried out at 310 K and 1 atm. Periodic boundary conditions were imposed in all three dimensions. The Particle Mesh Ewald (PME) method was used to calculate the long-range electrostatic interactions.[45] Following Cézard et al.,[36] a switching distance of 9 Å and a 10.5 Å cutoff was applied to the Lennard-Jones potential used to model van der Waals interactions. The SHAKE algorithm was used to constrain bonds involving hydrogen atoms.[46] A time step of 2 fs was used to integrate the equations of motion. Simulation snapshots were saved every 2 ps. The *collective variable* functionality of NAMD was used to apply a weak harmonic potential to restrict the center-of-mass motion of the diamide-linked γ-CD dimer in all simulations, while the motion of curcumin was unrestricted.

Thermodynamic Integration. Free energies of binding of curcumin inside the diamide-linked γ-CD dimers were calculated by alchemical free energy perturbation[47,48] using the thermodynamic integration options available within NAMD. The nonbonded interactions of curcumin were switched on or off linearly via a coupling parameter, λ.[49] The simulation was split into two components. The first component involved the 'creation' of curcumin inside a box of 3140 water molecules, by gradually increasing λ from 0 to 1 in 0.05 increments and running the simulation for 0.96 ns at each λ step. The second component was the 'destruction' of curcumin from within the diamide linked γ-CD dimer, by gradually decreasing λ from 1 to 0 in 0.05 decrements and running the simulation for 0.96 ns at each λ step. The 'creation' of curcumin in water was run 10 times from the same starting structure but with a different Langevin seed for each run. The 'destruction' of curcumin in the diamide-linked γ-CD dimer was run five times each from the three different starting orientations for each of succinamide- or urea-linked γ-CD dimer. The NAMD_ti.pl perl script was used to process the thermodynamic output for each run.[50] The difference between the average free energy of each process was used to determine the free energy of binding. The entire set of simulations was performed at 298, 310, 320, and 350 K for the urea-linked γ-CD dimer (although runs from only one starting structure were carried out at 350 K) and at 310 K for the succinamide-linked dimer.

Structural Analysis. To elucidate the way in which curcumin binds to the diamide-linked γ-CD dimers, various measurements were made with respect to planes passing through the cyclodextrin rings. The least-squares plane of best fit to each cyclodextrin was defined as that passing through the C1 and C4 carbons and the oxygen atoms in the glycosidic bonds linking glucopyranoside units (see Figure 1).[51,52] The first 5 ns of data were discarded for each simulation to remove potential bias due to starting configurations. The measurements of distances d and D (Figure 3a) involved taking the geometric centers of selections of atoms, excluding hydrogens. To measure the angle between the cyclodextrin planes, a reference coordinate system was defined based on two vectors (see Supporting Information for details). The first vector was the normal of the least-squares fit plane of CD 1. The second vector was between the geometric center of the linking glucopyranoside unit of CD 1 and the opposite glucopyranoside unit on CD 1. Using these two vectors an orthonormal coordinate system was defined for each frame. The projection of the normal vector to the best-fit plane to CD 2 onto the reference system defined by CD 1 gives the rotation of CD 2 with respect to CD 1 in three angles. The angle of interest between the CD planes is represented by θ in Figure 3a.

The orientation of curcumin with respect to the diamide-linked γ-CD dimer required a separate coordinate system. It is helpful to define the 'spine' of the dimer, which includes the linker and the two glucopyranoside units directly connected to it. A plane of best fit was made to the glucopyranoside units in the spine, excluding hydrogens. The normal vector to the plane of the spine was the first vector. The second vector was defined the same way as previously described. A plane of best fit was also obtained for all the atoms in curcumin, excluding hydrogens. The normal vector to the curcumin plane was projected onto the coordinate system, from which the 3 angles of curcumin with respect to the diamide linked γ-CD dimer were found. The angle of interest defining the orientation of curcumin with respect to the γ-CD dimer is represented by ϕ in

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Figure 3c. Details of the calculations used to characterize the structure of curcumin in the diamide-linked γ-CD dimers are given in the Supporting Information.

The structural analysis given in the Results and Discussion section below is for the equilibrium simulations carried out at 298 K. Structural distributions for the distance (d) between the geometric centers of curcumin and the cyclodextrin dimer, the angle (θ) between the planes of the cyclodextrins in the dimer, and the orientation angle (ϕ) of curcumin in the dimer from equilibrium simulations at 310 K of curcumin in the diamide-linked γ-cyclodextrin dimers are also given in Figures S11−S13 of the Supporting Information. The distributions at the higher temperature exhibit no major qualitative differences to those at the lower temperature.

RESULTS AND DISCUSSION

Binding Constant. Table 1 contains the simulated and previously measured experimental binding constants for the urea- and succinamide-linked γ-CD dimers at 310 K. The thermodynamic integration simulations for the binding constants are in good agreement with the experimental results.

Table 1. Simulated and Experimental 1:1 Curcumin/Dimer Binding Constants at 310 K

linker	$K_{simulation}$ (10^6 M^{-1})	$K_{experimental}$ (10^6 M^{-1})
urea	$1.6^{+3.2}_{-0.8}$	2.0 ± 0.1
succinamide	$11.3^{+20}_{-6.2}$	8.7 ± 0.4

The stronger binding constant for succinamide- compared with urea-linked γ-CD dimers can be rationalized as follows. The length of curcumin, as defined by the distance from the center-of-mass of one phenyl ring to the opposing phenyl ring is 12.25 ± 0.25 Å. The average distance between the geometric center of the γ-CDs in the diamide-linked γ-CD dimers is represented by D in Figure 3a and is given in Table 2. The results presented in Table 2 suggest two phenomena. First, the presence of curcumin limits the conformational freedom of the diamide-linked γ-CD dimers, leading to a smaller standard deviation of D. Second, the γ-CDs move closer to each other as a result of curcumin binding, which offers encapsulation and protection for curcumin against reactions with water. The length of the urea-linked γ-CD dimer is insufficient to accommodate the full length of curcumin and there is limited variation in D, suggesting that the conformation of the urea-linked γ-CD dimer is unable to adjust as well as the succinamide-linked dimer to accommodate the presence of curcumin, due to the shorter length and lack of rotatable bonds in the urea linker.

Table 2. Distance D between Geometric Centers of CDs in Dimers at 298 K

linker	D (curcumin) (Å)	D (empty) (Å)
urea	8.6 ± 0.6	9.9 ± 1.6
succinamide	10.16 ± 1.25	12.4 ± 2.7

To further rationalize the binding constant, the distance d between the geometric center of curcumin and that of the diamide-linked γ-CD dimer was measured (see Figure 3b). The distribution of d is shown in Figure 4 for both the urea- and succinamide-linked γ-CD dimers. Although both distributions have a maximum around 2 Å, the shapes of the distributions are clearly different. The broad distribution for the urea-linked γ-CD dimer indicates the absence of a tightly bound state for curcumin. In contrast, the distribution for the succinamide-linked γ-CD dimer is narrower and more sharply peaked and has two shoulders. These features suggest that there are likely two states for curcumin binding, one where curcumin is loosely bound inside the dimer and one where it is tightly bound. This tight binding can be attributed to the flexibility and additional length of the succinamide linker. The flexibility can be measured by observing the angle between the planes of the γ-CDs, θ, defined in Figure 3a. The flexibility allows the succinamide linked γ-CD dimer to encapsulate and trap curcumin more efficiently.

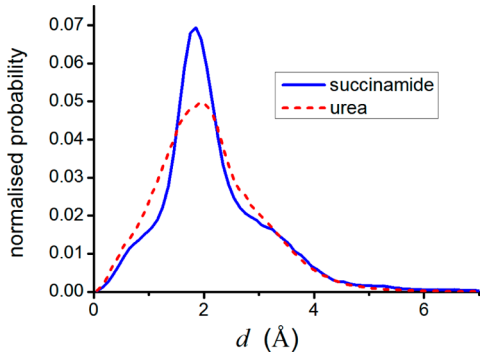

Figure 4. Probability distributions of distance d between geometric centers of curcumin and γ-CD dimer at 298 K.

Figure 5 shows the distributions of the angle θ between γ-CD planes for the succinamide- and urea-linked γ-CD dimers both with and without curcumin. Negative values of θ indicate the two γ-CDs closing in together, while positive values indicate the γ-CDs opening apart. In the absence of curcumin, the diamide-linked γ-CD dimers are relatively flexible, as demonstrated by the broad distributions extending well past the limits of the distributions in the presence of curcumin. The urea-linked γ-

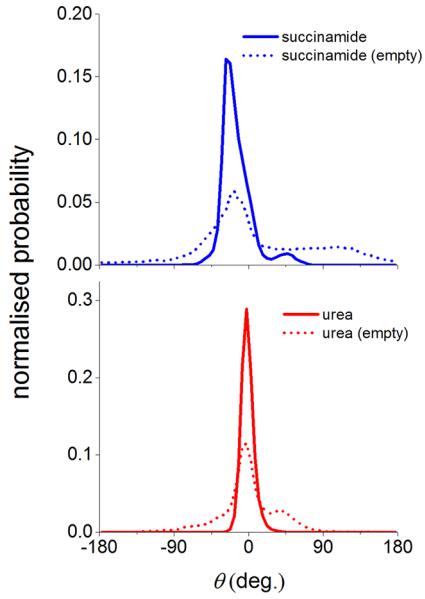

Figure 5. Probability distributions of the angle θ between the planes of γ-CDs in the diamide-linked γ-CD dimers for succinamide (top) and urea (bottom) linkers in the presence (solid) and absence (dotted) of curcumin at 298 K.

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CD dimer is relatively inflexible, with θ not exceeding $\pm 90°$ in the absence of curcumin. When curcumin is present in the urea-linked γ-CD dimer, the distribution of θ narrows and remains relatively centered on 0°. The lack of flexibility in the urea-linked γ-CD dimers is due to the lack of conformational freedom of the urea linker itself. There is also no significant shift in the equilibrium value of θ, with both the empty and full urea-linked γ-CD dimer remaining centered on 0°, indicating the γ-CDs are parallel to each other. The succinamide-linked γ-CD dimer is significantly more flexible, with θ ranging from -180 to $+180°$ in the absence of curcumin. In the presence of curcumin, the conformational freedom of the dimer is reduced significantly and the range of θ is limited to approximately $\pm 50°$. The equilibrium angle for the empty succinamide-linked γ-CD dimer sits at approximately $-20°$ indicating a slight closing together of the CDs. When curcumin is present inside the dimer, the equilibrium angle shifts approximately to $-25°$, demonstrating the CDs closing together further.

An Arrhenius plot of the binding constant of curcumin in the urea-linked γ-CD dimer is shown in Figure 6. From a linear fit

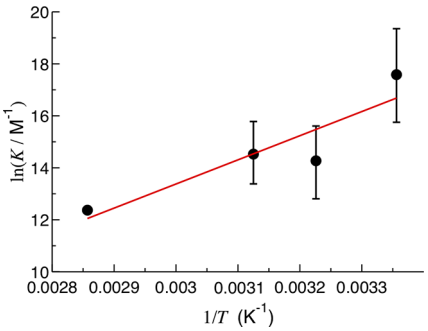

Figure 6. Arrhenius plot of binding constant of curcumin in the urea-linked γ-CD dimer (error bars are two standard errors).

to the temperature-dependence data, the enthalpy and entropy of binding were obtained as -77 ± 50 kJ.mol^{-1} and -120 ± 156 J·mol^{-1}.K^{-1} (error bars are two standard errors), respectively, indicating the binding process is favored enthalpically but disfavored entropically, although the latter result is not conclusive. This result is consistent with the expectation that displacement of water molecules in the hydrophobic core of the cyclodextrins by the hydrophobic curcumin, which increases the number of strong water−water hydrogen bonding interactions, should be exothermic. At the same time, the reduction in conformational freedom of the γ-CD dimer upon curcumin binding should decrease the entropy.

Stability of Curcumin in Diamide-Linked γ-CD Dimers. The accessibility of the keto−enol moiety of curcumin to water is crucial to rationalizing the stability of curcumin inside the diamide-linked γ-CD dimers. It has been shown previously that hydrolysis is a main mechanism for decomposition of curcumin in an aqueous environment.[14] The accessibility can be measured in terms of the orientation of the keto−enol moiety inside the diamide-linked γ-CD dimer and the distribution of water molecules around it. Figure 7 shows the distributions of the orientation angle ϕ of curcumin with respect to the γ-CD dimer (see Figure 3c) for the urea- and succinamide-linked dimers. The urea-linked γ-CD has a broad peak centered around 30°. It is important to note that three approximate starting configurations of ϕ at 0°, 90°, and 180° were used. The distribution for succinamide-linked γ-CD dimers shows sharp

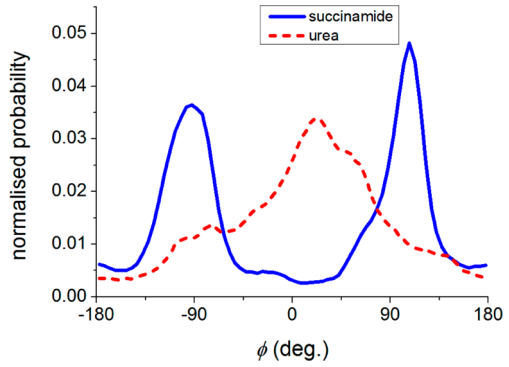

Figure 7. Probability distributions for curcumin orientation (ϕ) for γ-CD dimers linked by succinamide (solid) or urea (dashed) at 298 K.

peaks at 90°, indicating that curcumin is trapped perpendicular to the plane that passes from the side of the γ-CD dimer connected to the linker to the opposite side of the dimer. In most individual simulations, there was minimal interconversion between $+90°$ and $-90°$ over the 50 ns duration, suggesting a strong binding potential to trap curcumin in this orientation. The distribution for urea-linked γ-CD dimers shows a completely different behavior with a broad peak centered at $+30°$ when a distribution centered on 0° rather than $+30°$ might be expected. This counterintuitive result can be explained by the slight asymmetry in the curcumin keto−enol moiety and the chirality of the CDs in the diamide-linked γ-CD dimers. Curcumin was placed in the diamide-linked γ-CD dimers with the keto−enol moiety oriented the same way in all of the simulations. It is expected if the orientation of the keto−enol moiety were flipped, ϕ would be centered at $-30°$. The orientation of curcumin in the urea-linked γ-CD dimers is such that the keto−enol moiety interacts strongly with the linker. This orientation is likely to reduce the water accessibility of the keto−enol moiety and inhibit hydrolysis. The center of the distribution of ϕ in the urea-linked γ-CD dimer shifts toward 0° at 310 K (see Figure S13 in the Supporting Information), likely due to the weakening of the hydrogen bonds between curcumin and the urea linker at the higher temperature.

To measure the water accessibility of curcumin in both diamide-linked γ-CD dimers, the radial distribution functions (RDFs) were taken for the two oxygens of the keto−enol moiety and the oxygens of all water molecules, as shown in Figure 8. The distributions are nearly identical for the first 5 Å within error, except the magnitude in the first hydration shell,

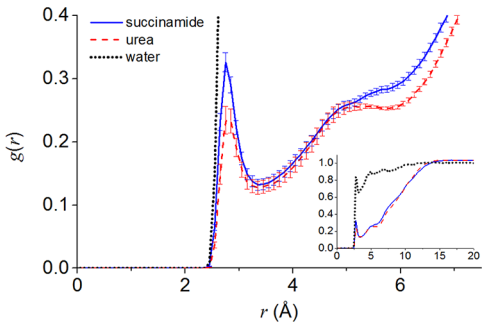

Figure 8. Radial distribution function (RDF) at 298 K for the keto−enol moiety of curcumin and water for curcumin encapsulated in γ-CD dimers linked by succinamide (solid) and urea (dashed) and curcumin in pure water (dotted). Inset shows the same RDFs extended to 20 Å.

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with the keto−enol moiety in the succinamide-linked γ-CD dimer slightly more likely to have a water molecule close to it than that in the urea-linked dimer. Also included is the same RDF for curcumin in pure water to demonstrate the large reduction of the water accessibility of curcumin upon encapsulation inside a diamide-linked γ-CD dimer. By integrating to the minimum after the first peak at 3.4 Å, the average number of water molecules found to be surrounding the keto−enol moiety of curcumin is 0.55, 0.66, and 2.53 for curcumin in the urea-linked γ-CD dimer, in the succinamide-linked dimer, and in pure water, respectively. The increased water availability in the succinamide-linked γ-CD dimers can be explained by the preferred orientation of curcumin at 90° to the linker inside it and the inability of the succinamide-linked dimer to remain completely closed owing to the length and flexibility of the linker. The increased stability of curcumin in the urea-linked γ-CD dimers is potentially explained by the protection of the keto−enol moiety by the linker due to the orientation of the moiety toward the linker, which decreases its water accessibility.

Further understanding of the accessibility of the keto−enol moiety of curcumin to water and the stability of curcumin in the diamide-linked γ-CD dimers can be obtained from a hydrogen-bond analysis of the keto−enol moiety. A hydrogen bonding analysis was performed using the HBonds plugin available in VMD,[43,44] in which a hydrogen bond was defined as existing between an atom with a hydrogen bonded to it (the donor) and another atom (the acceptor) if the donor−acceptor distance was less than 3.0 Å and the donor−hydrogen−acceptor angle was less than 20 degrees from collinear (the default settings for the HBonds plugin).

Hydrogen bonds were measured between the keto−enol moiety of curcumin and all water molecules, as well as all potential donor and acceptor atoms in the CD dimer. (In reality, the positions of the carbonyl and hydroxyl oxygens in the keto−enol moiety of curcumin readily swap due to intramolecular hydrogen transfer. This was not modeled in the classical MD simulations, in which the carbonyl and hydroxyl groups are distinct.) The occupancy of each unique hydrogen bond type was calculated as the percentage of trajectory configurations in which the bond was present in the simulation (the occupancy can therefore exceed 100% for an atom that can form two of the same type of hydrogen bond) and is given in Table 3.

The hydroxyl group was observed in the simulations only to act as a hydrogen bond donor to the carbonyl group. Hydrogen bonds between the keto−enol moiety and water molecules thus always involve the carbonyl or hydroxyl oxygen acting as an acceptor. It was found that the occupancy of carbonyl or hydroxyl oxygens in curcumin by hydrogen bonds with water is significantly higher when curcumin is not encapsulated in the CD dimer. In the simulations at 298 K, the water hydrogen-bond occupancy of the carbonyl oxygen was found to decrease from 43.6% in pure water to 23.0% and 13.8% for succinamide- and urea-linked dimers, respectively. The water hydrogen-bond occupancy of the hydroxyl oxygen is significantly lower, but follows the same trend, decreasing from 18.9% in pure water to 6.4% and 7.7% in the succinamide- and urea-linked dimers, respectively. The differences are less pronounced at 310 K, but the trend is the same. The average occupancy of a keto−enol oxygen is thus lowest for the urea-linked dimer, followed by the succinamide-linked dimer and then pure water, which agrees qualitatively the experimental stability of curcumin.

Instead of forming hydrogen bonds with water, the carbonyl oxygen of curcumin in the urea-linked dimer hydrogen bonds with the amine groups of the linker approximately 17% of the time. There are no such hydrogen bonds between the keto−enol oxygens of curcumin and the succinimade linker. This result suggests that the origin of the stability of curcumin in the urea-linked dimer compared with the succunamide-linked dimer could be preferential hydrogen bonding to the linker, which prevents hydrogen bonding to water and consequent hydrolysis.

Table 3. Hydrogen Bond Occupancies of Carbonyl and Hydroxyl Groups of the Keto−Enol Moiety of Curcumin in Urea- and Succinamide-Linked γ-CD Dimers and in Pure Water at 298 and 310 K

	urea (%)		succinamide (%)		water (%)	
hydrogen bond type	298 K	310 K	298 K	310 K	298 K	310 K
carbonyl−water	13.8	15.7	23.0	17.4	43.6	44.3
hydroxyl−water	7.7	6.0	6.4	6.6	18.9	19.2
carbonyl−amine	17.3	12.6	0	0	–	–
hydroxyl−amine	1.6	1.9	0	0	–	–

CONCLUSION

In this work, we simulated curcumin in urea-linked or succinamide-linked γ-CD dimer as well as the free diamide-linked γ-CD dimers in water. The binding constants were calculated from simulations by thermodynamic integration and showed excellent agreement with previously found experimental results. The distance d between the geometric centers of curcumin and the succinamide-linked γ-CD dimer is shorter than that between curcumin and the urea-linked γ-CD dimer, indicating a stronger binding of curcumin to the former. The two CD annuli were observed to close together upon binding of curcumin, as measured by the distance D between the geometric centers of the CDs. This phenomenon was further confirmed by measuring the angle θ between the planes of the CDs, which revealed that the more flexible succinamide linker closes together more in the presence of curcumin. The urea linker is relatively inflexible and did not exhibit a significant change in θ. The orientation of curcumin inside the dimers with respect to the linkers, as measured by ϕ, revealed that curcumin prefers to orient its keto−enol moiety toward the linker in the urea linked dimer (ϕ = 0°), whereas it adopts an orientation with ϕ = 90° in the succinamide-linked dimer. Finally, an analysis of the distribution of water molecules and water hydrogen bonds around the curcumin keto−enol moiety showed a difference in water accessibility, indicating that the keto−enol moiety is more exposed with the succinamide linker than with urea. These results help to rationalize the behavior of curcumin in the succinamide- and urea-linked dimers.

ASSOCIATED CONTENT

Supporting Information

Measurements of d, D, θ, and ϕ over the duration of a single simulation for both diamide-linked dimers at 298 K. Definition of coordinate systems and angular coordinates, α, θ, and ϕ. Distribution of α for both diamide-linked dimers. Distribution of d, θ, and ϕ for both diamide-linked dimers at 310 K. This material is available free of charge via the Internet at http://pubs.acs.org.

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