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Exploring the Energy Landscapes of Cyclic Tetrapeptides with Discrete Path Sampling

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Supporting Information

ABSTRACT: Cyclic tetrapeptides are an important class of biologically active molecules that exhibit interesting conformational dynamics, with slow interconversion of several different structures. We present calculations on their energy landscapes using discrete path sampling. In acyclic peptides and large cyclic peptides, isomers containing cis-peptide groups are much less stable than the all-trans isomers and separated from them by large barriers. Strain in small cyclic peptides causes the cis and trans isomers to be closer in energy and separated by much lower barriers. If D-amino acids or proline residues are introduced, isomers containing cis-peptides become more stable than the all-trans structures. We also show that changing the polarity of the solvent has a significant effect on the energy landscapes of cyclic tetrapeptides, causing changes in the orientations of the peptide groups and in the degree of intramolecular hydrogen bonding.

1. INTRODUCTION

Cyclic peptides have a number of properties that make them useful for biomedical applications. The constraints of cyclization give them a smaller accessible conformational space than acyclic peptides, which leads to a smaller loss of entropy when they bind to a receptor.[1] They are also very stable because they are not broken down by exopeptidases, which digest peptides by removing residues from the end of the peptide chain.[1] Cyclic peptides of all sizes are biologically active, starting from cyclic dipeptides, which are known as diketopiperazines.[2] Interesting cyclic tetrapeptides include the antitumor agent trapoxin,[3] the antimalarial apicidin,[4] and the phytotoxic tentoxin.[5] There are also many examples of biologically active cyclic peptides containing five,[6] six,[7,8] or more[1,7] peptide groups.

Understanding the energy landscapes of cyclic peptides will account for their conformational dynamics and provide some insight into their biological activity. Small cyclic peptides have very different conformational behavior to acyclic peptides, most significantly with respect to cis/trans isomerization of the peptide groups. Cyclic dipeptides have both peptide bonds in the cis conformation because this is the only configuration that allows for closure of the ring. All of the known experimental structures for cyclic tripeptides have all-cis conformations, but ab initio calculations on cyclic triglycine show that the all-cis and trans−cis−cis isomers are close in energy.[9] Many cyclic tetrapeptides exhibit interesting conformational dynamics with slow interconversion of several structures and competition between the cis and trans isomers of the peptide groups.[5,10−12] As the size of the ring increases, the cis/trans ratio in cyclic peptides approaches that seen in acyclic peptides.[12]

The conformations of cyclic peptides have been explored with a variety of computational techniques. The most stable conformers of several cyclic tetrapeptides have been located either by systematic[11] or Monte Carlo[12] searches. The barriers to isomerization have been studied by molecular dynamics.[5,12] Ab initio methods have been used to study the pathways for conversion between a small number of minima in cyclic tri-[9] and hexapeptides.[13,14] Many larger cyclic peptides, comprising up to ten residues, have also been studied with molecular dynamics.[6,13−17] Other methods to generate cyclic peptide conformers include dihedral angle sampling,[18] distance geometry methods,[19] and the NcCYP method,[20] which uses a combination of coarse-grained and all atom models to generate the conformers of large cysteine-rich cyclic peptides.

Cyclic tetrapeptides have energy landscapes containing a few hundred minima. This is a small enough conformational space for discrete path sampling[21−23] to sample all of the physically relevant minima and transition states. In this study, we present a detailed analysis of the energy landscape of cyclo-[Gly_4] and compare this to some larger and less strained cyclic peptides as well as an acyclic peptide. We then study a number of peptides where some of the glycine residues are replaced by the L- and D-isomers of alanine, to study the effect of side chains on the backbone of the peptide without the additional expense of large flexible side chains. We also consider substitution by proline, in which the cis and trans isomers are much closer in energy than in other amino acids[24] and which has been shown to promote structural features like β-turns.[25]

2. METHODS

We examine the energy landscapes of several cyclic tetrapeptides, the simplest of which is cyclo-[Gly_4]. We compare this energy landscape with the larger cyclic peptides cyclo-[Gly_5] and cyclo-[Gly_6] and the methyl-capped Ace-Gly_3NMe, which contains four peptide groups and is the acyclic peptide that most closely resembles cyclo-[Gly_4]. We have

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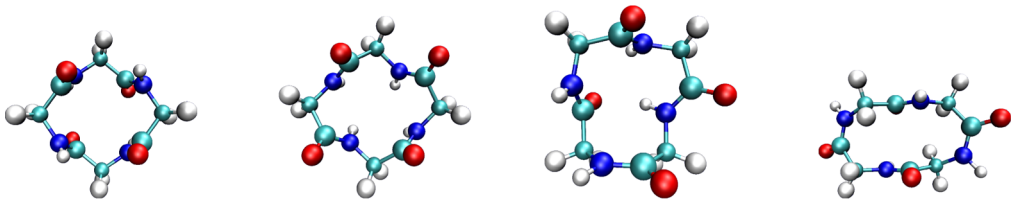

Figure 1. Selected conformers of cyclo-[Gly_4] optimized with the AMBER ff03 potential in water.

constructed energy landscapes for all of the cyclic tetrapeptides where one or more of the glycine residues have been replaced by alanine residues (cyclo-[$AlaGly_3$], cyclo-[$(AlaGly)_2$], cyclo-[Ala_2Gly_2], cyclo-[Ala_3Gly], and cyclo-[Ala_4]). We also study the conformations of cyclic peptides containing both D- and L-peptides by looking at cyclo-[D-AlaGly L-AlaGly] and cyclo-[(D-Ala L-Ala)$_2$]. Many biologically active tetrapeptides contain at least one proline residue,[11] and we study cyclo-[Gly_3Pro], cyclo-[$(GlyPro)_2$], and the larger cyclic peptides cyclo-[Gly_4Pro] and cyclo-[Gly_5Pro].

Discrete path sampling calculations were performed with PATHSAMPLE.[26] The initial minima for the discrete path sampling calculations were located with the basin-hopping algorithm[27] as implemented in GMIN.[28] Initially, pairs of minima for connection were selected with the missing connection algorithm.[29] Transition states were located using the doubly nudged elastic band method[30] with interpolation between end points using a Cartesian coordinate interpolation scheme[30,31] and optimized by hybrid eigenvector following[32−34] in OPTIM.[35] Later, pairs of minima for connection were selected using the UNTRAP method to remove artificial frustration.[36] These networks of stationary points are visualized as disconnectivity graphs.[37,38] We present only the most important disconnectivity graphs here, with the graphs for all the other cyclic peptides discussed in this paper available as Supporting Information. Some of the cyclic peptides studied here are highly symmetrical, with many symmetry equivalent minima and transition states. In the disconnectivity graphs for these compounds, all of the symmetry equivalent isomers are collected together. In the most symmetrical compounds this gives a much smaller number of stationary points. For example, cyclo-[$AlaGly_3$] has 369 minima accessible via transition states less than 30 kcal mol^{-1} above the global minimum, but cyclo-[Gly_4] has just 54 symmetry unique minima. Construction of the database of stationary points for a typical unsymmetrical cyclic tetrapeptide, such as cyclo-[$AlaGly_3$], requires about 24 h walltime on four cores of an Intel Xeon E5405 CPU with a clock speed of 2.0 GHz.

The energies of all structures were evaluated using the AMBER ff03 force field.[39−41] Solvent effects were modeled using the GB/SA implicit solvation method.[42,43] Topology files for cyclic peptides prepared using the AMBER LEaP program[44] give small energy differences between structures that should be degenerate. This problem was resolved by reordering the atoms defining the improper torsion angles at the point of ring closure.[45] Cyclic tetrapeptides are strained molecules, and we must check that the AMBER force field accurately generates the relative energies of the stationary points. The smallest cyclic tetrapeptide, cyclo-[Gly_4], was chosen for higher level calculations because it is the least computationally demanding in terms of the number of stationary points and the size of each calculation. The energies of all stationary points on the cyclo-[Gly_4] landscape were re-evaluated by single-point density functional theory (DFT) calculations at the B3LYP/6-31G* level as implemented in NWChem.[46] Additionally, the structures of key minima from the AMBER potential energy surface were reoptimized at the B3LYP/6-31G* level. Solvation in water was modeled with the COSMO method.[47]

When naming cyclic peptides, we use the same conventions as Loiseau.[11] A cyclic tetrapeptide can be described by up to four different sequences because the starting point for the sequence in a cyclic peptide is arbitrary. We assign the first position in the sequence to the amino acid that is first alphabetically. When labeling conformers, the plane of the ring is defined by the mean plane of the four α-carbon atoms. The molecule is oriented with the ring running clockwise, and the peptide groups are labeled as up (u) or down (d) from the position of the peptide oxygen relative to the plane. Each minimum is labeled by the sequence of cis/trans and up/down isomers (e.g., ctct-uudd). Note that sometimes multiple structures can be described by the same label.

3. RESULTS AND DISCUSSION

3.1. DFT Calculations. In the gas phase, both the B3LYP and AMBER calculations agree on the S_4 symmetrical tttt-dudu conformer as the global minimum (Figure 1a) of cyclo-[Gly_4]. The energies of all the minima relative to the global minimum show a good correlation between both methods (Figure 2a). However, the energy separations in AMBER are slightly lower than those calculated with B3LYP. The agreement is also good for the transition states up to 30 kcal mol^{-1} above the global minimum. Reoptimization of the tttt-udud conformer at the B3LYP/6-31G* level leads to no structural change except for a small increase in the pyramidalization of the nitrogen atoms in the peptide bonds.

In the aqueous phase, the agreement between the AMBER and B3LYP energies is less good but still acceptable (Figure 2b). The two methods disagree on the ordering of the most stable structures. In both cases, the global minimum is all-trans. With B3LYP, the global minimum is the dudu isomer. However, AMBER prefers the C_2 symmetrical uuuu structure, with two of the peptide groups almost perpendicular to the plane of the ring and the other two tilted outward by 15° (Figure 1b). With the AMBER force field, the uuuu conformer is a higher-order saddle point on the potential energy surface in vacuo. Breaking the symmetry of this structure followed by minimization leads to the duuu isomer. The uuuu isomer is a minimum on the B3LYP gas-phase potential energy surface, but it lies 29 kcal mol^{-1} above the dudu global minimum.

3.2. Cyclization. The relative energies of the cis- and trans-peptides and the barriers between them are strongly dependent on the size of the cyclic peptide ring (Table 1). In the acyclic peptide, the most stable isomer containing a cis-peptide is 4.9 kcal mol^{-1} above the all-trans global minimum and separated

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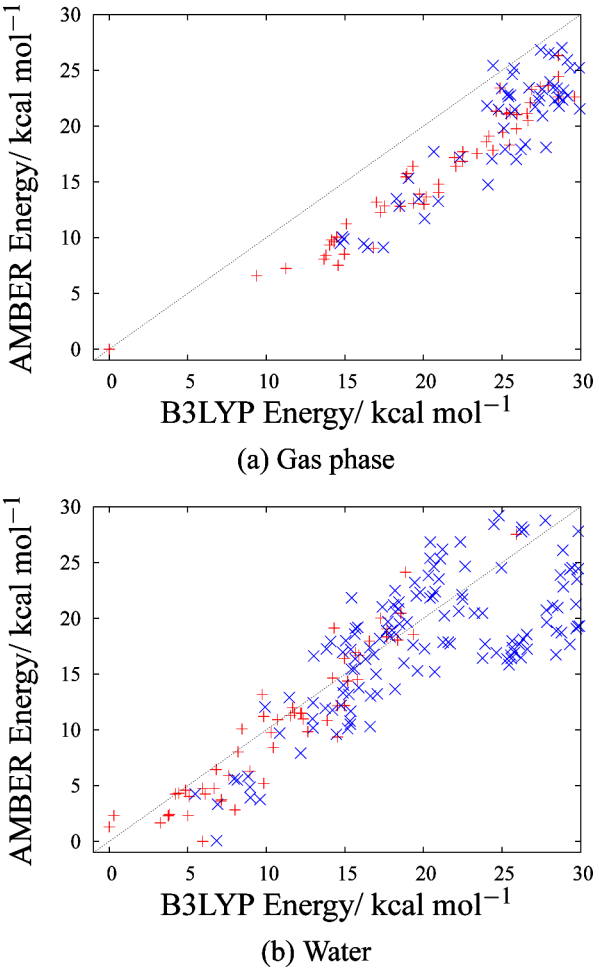

Figure 2. Relative energies of the stationary points on the cyclo-[Gly_4] energy landscape calculated at the B3LYP/6-31G* and AMBER/ff03 levels. Points in red are minima, and points in blue are transition states.

Table 1. Energies of the Most Stable Structures Containing cis-Peptides in Some Cyclic and Acyclic Polyglycines Relative to the All-Trans Global Minima[a]

	number of cis-peptides				
sequence	1	2	3	4	trans/cis barrier
cyclo-[Gly_4]	2.3	4.2	9.7	14.7	16.4
cyclo-[Gly_5]	3.5	7.9	13.9	20.0	16.8
cyclo-[Gly_6]	4.8	9.6	15.2	20.3	21.2
$AceGly_3NMe$	4.9	10.0	14.1	18.6	21.5

[a]Also shown are the energies of the lowest trans−cis transition states. All energies are in kcal mol^{-1} and calculated with the AMBER ff03 force field in water.

from it by a barrier of 21.5 kcal mol^{-1} (Figure 3). In the unstrained cyclic hexapeptide cyclo-[Gly_6], the energies and barriers for the trans−cis rearrangement are almost identical to those for the acyclic peptide. Reducing the size of the ring to cyclo-[Gly_5] gives a smaller difference of 3.5 kcal mol^{-1} between the trans and cis isomers and a much smaller barrier to their interconversion of 16.8 kcal mol^{-1}. In the cyclic tetrapeptide cyclo-[Gly_4] the energy required to introduce a cis-peptide and the barriers to trans−cis conversion are even lower (Figure 4). No conformation of the twelve-membered ring can satisfy all of the preferred values of its component bond angles

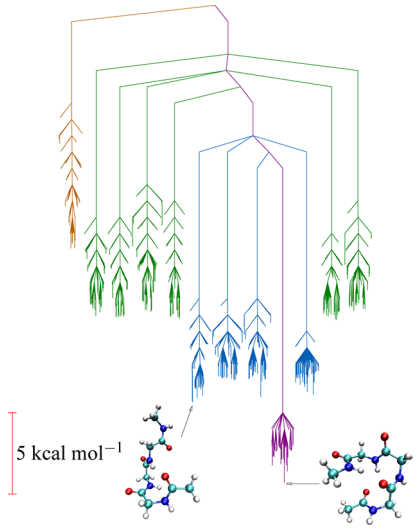

Figure 3. Disconnectivity graph showing the energy landscape of $Ace-Gly_3$-NMe in water including the 841 minima and 5786 transition states accessible via transition states lower than 30 kcal mol^{-1} from the global minimum. Minima are colored by the number of trans-peptide groups from orange (1) to purple (4).

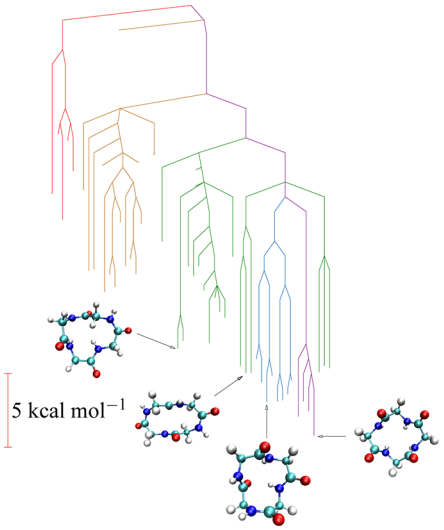

Figure 4. Disconnectivity graph showing the energy landscape of cyclo-[Gly_4] in water including the 54 minima and 255 transition states accessible via transition states lower than 30 kcal mol^{-1} from the global minimum. Minima are colored by the number of trans-peptide groups from red (0) to purple (4).

and torsions, and this strain is responsible for lowering the barriers to cis/trans isomerization. The energies and barriers associated with introducing two or more cis bonds into cyclo-[Gly_4] are also much lower than in the acyclic peptide. When two cis-peptides are present, the ctct arrangement is more stable than cctt.

In the acyclic peptide, the barriers to rotation of the ϕ and ψ torsion angles in the peptide backbone tend to be less than 5 kcal mol^{-1} (Figure 3). These correspond to the transitions between the up and down isomers in the cyclic peptides, which require concerted motion of several torsional angles and vary over a much wider range of energies. In the tttt arrangement of cyclo-[Gly_4], these barriers are all smaller than 5 kcal mol^{-1}. However, in conformers with at least one cis-peptide

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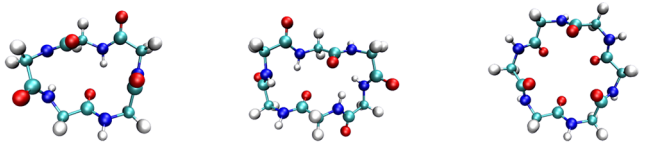
arrangement many of the barriers to rotation of the single bonds are much larger. In the case of the ctct isomers some of these barriers are within 1−2 kcal mol^{-1} of the cis/trans barriers. These larger barriers occur because up/down isomerization of a cis-peptide requires substantial deformation of the rest of the molecule.

Hydrogen bonding plays a key role in stabilizing many peptide structures. The global minimum structure for AceGly$_3$NMe has a hydrogen bond between the two peptide groups at the ends of the chain (Figure 3). The global minimum of the cyclic hexapeptide (Figure 5b) and many of



(a) cyclo-[Gly$_5$] (b) cyclo-[Gly$_6$] (c) cyclo-[Gly$_6$]

Figure 5. Selected conformers of cyclo-[Gly$_5$] and cyclo-[Gly$_6$] optimized with the AMBER ff03 potential in water.

the other low-lying structures contain two transannular hydrogen bonds. However, the most stable structure with no hydrogen bonds (Figure 5c) is only 0.3 kcal mol^{-1} less stable then the global minimum. A single hydrogen bond is present in all of the low-lying structures in cyclo-[Gly$_5$]. In cyclic tetrapeptides, the constraints of the ring make it difficult to form hydrogen bonds without introducing strain into the peptide backbone. In the aqueous phase, the tttt-uuuu global minimum contains no hydrogen bonds, and hydrogen bonding only makes a small contribution to the other tttt structures. As will be discussed in the next section, hydrogen bonding becomes more important to the tttt structures in less polar solvents. If a single cis-peptide bond is introduced, the two peptide groups on either side of this are well aligned to form a hydrogen bond (Figure 1c). In the ctct structures, hydrogen bonding becomes impossible because the two cis-peptides point outward in the plane of the ring while the two trans-peptides have to lie perpendicular to the plane of the ring (Figure 1d).

3.3. Solvation. In a low dielectric medium, the dudu conformer of cyclo-[Gly$_4$] is the most stable by a significant margin (Figure 6). The dipoles of the four peptide groups are aligned so that this conformer has no dipole moment.

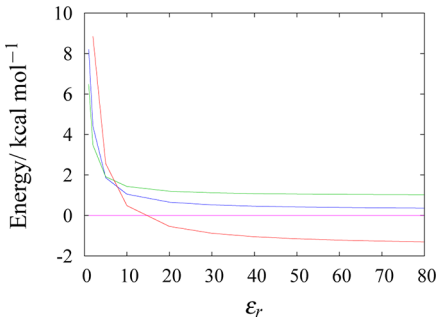


Figure 6. Relative energies of the up/down isomers of tttt cyclo-[Gly$_4$] as a function of the solvent dielectric constant. The lines represent the uuuu (red), duuu (green), dduu (blue), and dudu (pink) conformations. Where multiple structures have the same u/d configuration only the lowest is shown.

Changing the polarity of the solvent distorts this structure due to changes in the hydrogen bonding. In the gas phase each peptide group is hydrogen bonded to the peptide groups at positions i−1 and i+1, but these hydrogen bonds are rather bent with N−H−O angles of 134° and an H−O distance of 2.3 Å. In the aqueous phase the hydrogen bonding becomes much weaker, and the ring relaxes to place the H and O atoms 2.8 Å apart with an N−H−O angle of 116°. Increasing the polarity of the solvent stabilizes the polar uuuu isomer, and it is the global minimum for values of ε_r > 15. The duuu and dduu isomers both have small dipole moments and so are stabilized by increasing the polarity of the solvent but not to the same extent as the uuuu isomer. In nonpolar solvents, each of these isomers splits into two minima stabilized by different patterns of hydrogen bonds. The ^{1}H and ^{13}C NMR spectra of cyclo-[Gly$_4$] have been recorded in trifluoroacetic acid (ε_r = 8.4)[48] and show that all four peptide groups are equivalent.[49] The proposed structure was tttt-dudu, which is consistent with the calculations presented here.

The relative stabilities of the conformers can be understood in terms of the components of the AMBER energy (Table 2).

Table 2. Components of the AMBER ff03 Energy (in kcal mol^{-1}) for Key Conformers of cyclo-[Gly$_4$]^a

conformer	strain	steric	electrostatic	solvation
tttt-dudu$_g$	50.5	−0.2	−19.1	0.0
tttt-dudu$_{aq}$	49.2	−2.6	−12.6	−19.9
tttt-uuuu	46.3	−1.0	8.2	−40.7
tttt-dduu	49.6	−2.0	−8.1	−24.5
cttt-uduu	50.0	−0.9	−8.0	−26.1
ctct-dduu	51.5	−0.4	−11.1	−23.0

^{a}The components for the gas-phase tttt-dudu isomer are included for comparison. The strain energy includes the bond stretching, angle and torsion terms in the potential. The steric energy includes all nonbonded terms except for the electrostatic terms.

The tttt-udud isomer has the lowest electrostatic energy because of a favorable alignment of the dipoles of the four peptide groups. If the tttt-udud isomer is moved from polar to nonpolar conditions, the structure becomes distorted by the shortening of the hydrogen bonds. This gives a more favorable electrostatic component of the energy at the expense of worse steric and strain components. The tttt-uuuu isomer has a high electrostatic energy because the four dipoles are almost parallel. However, solvation in water stabilizes conformers with a large dipole moment, such as tttt-uuuu over those with no dipole. The tttt-uuuu conformer has the lowest strain energy, and introduction of cis-peptide bonds leads to increased strain.

3.4. L- and D-Alanine Containing Tetrapeptides. If one of the glycine residues in cyclo-[Gly$_4$] is replaced with an alanine residue to make cyclo-[AlaGly$_3$], then the four peptide groups are nonequivalent, which gives a much larger number of stationary points (Figure 7). The global minimum has a tttt-dddd conformation similar to that seen in cyclo-[Gly$_4$]. The relative energies of minima containing cis-peptide bonds are similar to those seen in cyclo-[Gly$_4$] (Table 3) as are the barriers to cis/trans transitions (Figure 7). If a second alanine residue is introduced to make cyclo-[(AlaGly)$_2$] or [Ala$_2$Gly$_2$], the tttt-dddd conformer is still the global minimum (Table 3). The relative energies of the lowest cis isomers and the barriers linking them to the global minimum are similar to those seen in

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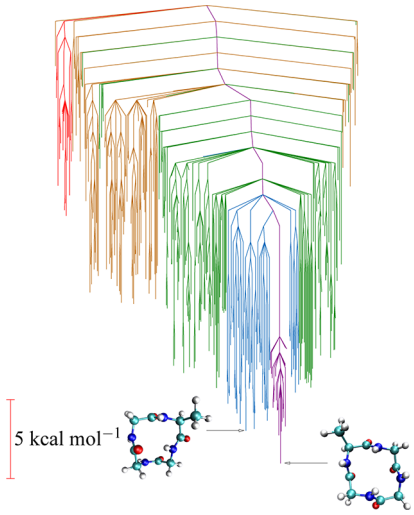

Figure 7. Disconnectivity graph showing the energy landscape of cyclo-[$AlaGly_3$] in water including the 369 minima and 2708 transition states accessible via transition states lower than 30 kcal mol^{-1} from the global minimum. Minima are colored by the number of trans-peptide groups from red (0) to purple (4).

Table 3. Relative Energies (in kcal mol^{-1}) of the Lowest Minima for Each Arrangement of cis/trans-Peptide Groups in Some Cyclic Tetrapeptides[a]

	number of cis-peptides				
sequence	0	1	2	3	4
cyclo-[Gly_4]	0.0	2.3	4.2	9.7	14.7
cyclo-[$AlaGly_3$]	0.0	2.1	4.5	10.1	15.7
cyclo-[$(AlaGly)_2$]	0.0	3.0	4.8	11.2	16.9
cyclo-[Ala_2Gly_2]	0.0	2.2	5.0	9.6	16.3
cyclo-[Ala_3Gly]	0.0	3.3	5.2	10.3	17.4
cyclo-[Ala_4]	0.0	4.9	5.0	11.3	18.3
cyclo-[D-AlaGly L-AlaGly]	0.9	0.0	3.7	8.5	15.3
cyclo-[$(D\text{-Ala L-Ala})_2$]	0.2	0.0	1.8	13.8	20.7
cyclo-[$ProGly_3$]	3.3	0.0	2.3	7.2	12.8
cyclo-[$(ProGly)_2$]	5.0	2.0	0.0	4.9	12.9

[a]All energies are calculated with the AMBER ff03 force field in water.

cyclo-[Gly_4]. However, some of the barriers to up−down isomerization are larger than those for cis−trans isomerization.

If a third alanine residue is added, the relative energies of the tttt and ctct isomers are unchanged, but the cttt isomer becomes less stable. This trend continues with cyclo-[Ala_4], where the most stable cttt and ctct conformers are of similar energy. The electrostatic energy contributions from the interactions of the peptide dipole moments are important in determining the relative energies of the structures (Table 4), with the polar dddd structure the most stable in water and the nonpolar udud structure the most stable in vacuo. NMR spectra of cyclo-[Ala_4] show a mixture of several stable conformations.[11] In water, four conformers are observed, with three of these merging at higher temperatures. It is possible that the three signals that merge correspond to the tttt isomer and two cttt isomers, because the downhill barriers separating them are relatively small (Figure 8). The ctct isomers are separated by larger downhill barriers and could be the source of the signals that do not merge at high temperatures. In $CDCl_3$, only the tttt and ctct isomers are observed, with none of the cttt isomer. The disconnectivity graphs here show the potential energy surface,

Table 4. Components of the AMBER ff03 Energy (in kcal mol^{-1}) for Key Conformers of cyclo-[Ala_4] in Water[a]

conformer	strain	steric	electrostatic	solvation
tttt-dddd	42.3	−1.6	17.4	−45.0
tttt-dudu	51.6	−0.4	−10.6	−18.0
tttt-dduu	47.7	−0.7	−0.7	−27.7
cttt-uduu	46.8	−1.6	8.4	−35.6
ctct-dduu	47.3	−1.0	−0.3	−27.9

[a]The strain energy includes the bond stretching, angle and torsion terms in the potential. The steric energy includes all nonbonded terms except for the electrostatic terms.

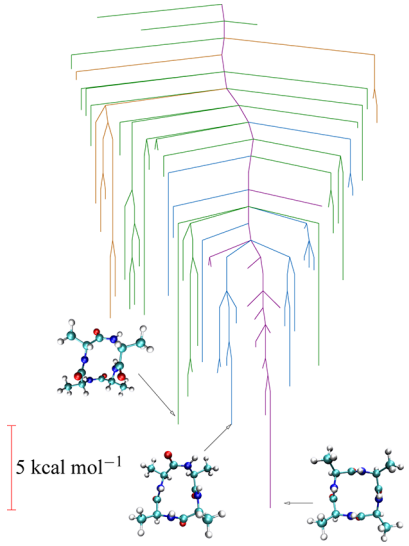

Figure 8. Disconnectivity graph showing the energy landscape of cyclo-[Ala_4] in water including the 67 minima and 199 transition states accessible via transition states lower than 30 kcal mol^{-1} from the global minimum. Minima are colored by the number of trans-peptide groups from orange (1) to purple (4).

but free energy disconnectivity graphs may be more appropriate for studying the relative populations of each conformer.[50−52]

The introduction of D-amino acid residues leads to an increase in the amount of strain. The most stable conformer of cyclo-[D-AlaGly-L-AlaGly] is 2.3 kcal mol^{-1} less stable than the most stable conformer of cyclo-[$(AlaGly)_2$]. The global minimum structure has a single cis-peptide bond. This allows the ring to adopt a chairlike structure, with one of the methyl groups axial to the ring. The most stable tttt structure is tttt-dudd, which is 0.9 kcal mol^{-1} higher in energy. The tttt-dddd isomer, which is the global minimum in water for all the previously discussed peptides, is 1.7 kcal mol^{-1} above the global minimum. This conformer places one of the alanine methyl groups close to two of the peptide oxygen atoms below the plane of the ring, which accounts for its destabilization.

The global minimum of cyclo-[$(D\text{-Ala-L-Ala})_2$] is 1.7 kcal mol^{-1} less stable than the global minimum of cyclo-[Ala_4]. The most stable conformers with tttt, cttt, and ctct arrangements are all close in energy (Figure 9). The most stable tttt isomer has the peptide groups in an udud arrangement. The tttt-uuuu isomer is 5.7 kcal mol^{-1} above the global minimum. Due to steric clashes, conformers containing three or four cis-peptides are less stable in this compound than in all other cyclic tetrapeptides (Table 3).

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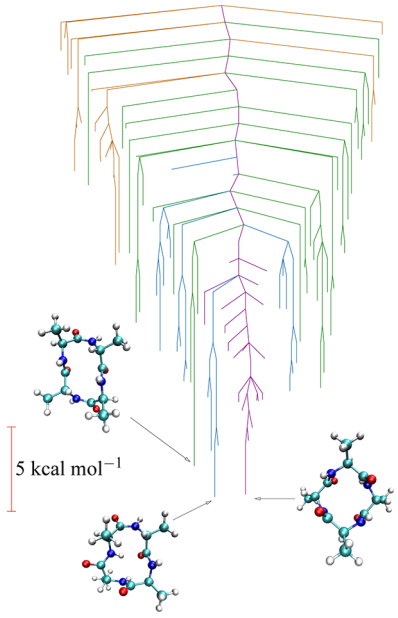

Figure 9. Disconnectivity graph showing the energy landscape of cyclo-[(D-Ala-L-Ala)$_2$] in water including the 116 minima and 524 transition states accessible via transition states lower than 30 kcal mol^{-1} from the global minimum. Minima are colored by the number of trans-peptide groups from orange (1) to purple (4).

3.5. Proline Containing Tetrapeptides. In the proline containing cyclic tetrapeptides, there are groups of minima with the same pattern of cis/trans and up/down configurations separated by barriers of 2−3 kcal mol^{-1}. These minima correspond to distortions of the five-membered rings in the proline residues. Due to the conformational restriction imposed by the proline ring, simple up/down isomerizations of the amide group attached to this ring do not occur.

The global minimum structure for cyclo-[Gly$_3$Pro] has the peptide group in the proline residue in the cis conformation (Figure 10). If the proline group is in the trans conformation the ring cannot be closed without at least one of the other peptide groups being in an unfavorable conformation. In this cyclic tetrapeptide, the proline residue strongly favors a cis conformation, with several ctct conformers below the most stable one containing a trans proline. However, the barriers to cis−trans isomerization of the proline residue are smaller than those for the glycine residues.

As the size of the ring in the cyclic peptide increases, the trans isomer of the proline residue becomes more stable (Table 5). The relative energies of the conformers containing cis and trans proline groups in cyclo-[Gly$_5$Pro] are very close. This trend is similar to that seen for the glycine residues in cyclic polyglicines of different sizes. It is noteworthy that the barrier to the cis−trans isomerization of the peptide bond preceding the proline ring does not vary much with the size of the cyclic peptide ring. The global minimum for cyclo-[(GlyPro)$_2$] adopts a ctct-udud arrangement. The lowest all-trans isomer is 5.0 kcal mol^{-1} above the global minimum and slightly above one ccct conformer (Table 3). The predicted preference for a ctct structure is consistent with the available NMR spectra[53,54] and in agreement with the known crystal structure[55] of cyclo-[(GlyPro)$_2$].

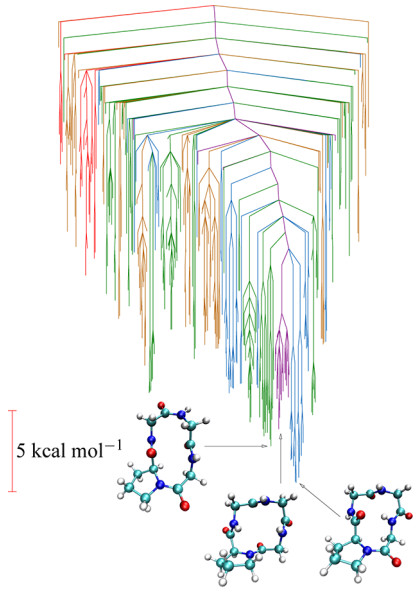

Figure 10. Disconnectivity graph showing the energy landscape of cyclo-[Gly$_3$Pro] in water including the 358 minima and 2472 transition states accessible via transition states lower than 30 kcal mol^{-1} from the global minimum. Minima are colored by the number of trans-peptide groups from red (0) to purple (4).

Table 5. Relative Energies (in kcal mol^{-1}) of the Lowest Minima for Each Arrangement of cis/trans-Peptide Groups in Some cyclo-[Gly$_n$Pro][a]

	number of cis-peptides		
sequence	0	1	trans/cis barrier
cyclo-[Gly$_3$Pro]	3.0	0.0	14.7
cyclo-[Gly$_4$Pro]	1.4	0.0	13.6
cyclo-[Gly$_5$Pro]	0.0	0.3	15.4

[a]Also shown are the energies of the lowest trans−cis transition states. All energies are in kcal mol^{-1} as calculated with the AMBER ff03 force field in water and are relative to the global minimum for that cyclic peptide.

4. CONCLUSIONS

The energy landscapes of small cyclic peptides are very different from those of larger cyclic peptides and acyclic peptides. As the size of the ring decreases, isomers containing cis-peptide groups become more stable, and the barriers to trans−cis isomerization become smaller. In cyclo-[Gly$_4$], the simplest cyclic tetrapeptide, the global minimum is all-trans, and the energy of the molecule increases when the number of cis-peptide bonds increases. Substituting one or two of these glycine residues with alanine gives a much larger number of minima due to the lower symmetry of these molecules, but the energy differences and barriers between these minima are similar to those seen in cyclo-[Gly$_4$]. Introducing more alanine residues leads to higher barriers and destabilization of some minima due to steric crowding. The peptide bonds preceding proline groups have a much smaller preference for the trans conformation in cyclic hexapeptides and adopt the cis conformation in smaller systems.

Solvation has a substantial effect on the energy landscapes of cyclic tetrapeptides. In nonpolar solvents conformers with no net dipole moment, such as the udud isomers, are the most stable. As the polarity of the solvent increases, isomers with

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large dipole moments are stabilized and become competitive with, or more stable than, the nonpolar ududd conformers. Due to the small ring size, the structures including hydrogen bonds have strained geometries. Such structures are only stable in nonpolar conditions where the strength of the hydrogen bond overcomes this strain.

We have presented the potential energy landscapes of several cyclic tetrapeptides. In the future, we must consider free energy landscapes[50−52] to obtain a full picture of the conformational dynamics of these molecules. Here, we have only considered cyclic tetrapeptides comprising four types of amino acid residue. It is likely that other natural or unnatural amino acids will influence the conformations of cyclic peptides in different ways, and these will also be the focus of future studies.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Disconnectivity graphs for all the cyclic peptides discussed in this paper. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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