Left-Handed and Ambidextrous Helices in the Gas Phase

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We have used ion mobility mass spectrometry to study the effect of D-residues on helix formation in unsolvated alanine-based peptides. The right-handed helix of $Ac-A_{15}K + H^+$ is significantly disrupted when five or more of the natural L-residues are randomly replaced with D-residues. On the other hand, when a block of L-residues is replaced with D-residues, an unusual ambidextrous structure with helical segments of opposite chirality is formed. A peptide with all D-residues forms a left-handed helix.

Introduction

The right-handed α-helix is the most commonly occurring secondary structure element in proteins. The right-handed twist of the helix is determined by the chirality of the constituent amino acids. The naturally occurring L-amino acids produce a right-handed twist, while the D-amino acids produce a left-handed one. The effect of amino acid chirality on the handedness of polypeptide helices was first noted by Pauling.[1] The functionality of many biological molecules is determined by chirality. Although the L-form is the predominant enantiomer found in nature, there are examples of the occurrence of D-amino acids. Some bacterial peptides and the amphibian skin peptides dermorphin and deltorphin contain D-amino acids.[2,3] D-amino acids in peptides and proteins are believed to be formed by posttranslational modifications,[4] and their presence increases proteolytic stability.[5,6] It has been shown that a D,L-amphipathic helix motif preferentially kills tumor cells over normal cells.[7]

When the L-amino acids in a helical peptide are replaced by the corresponding D-amino acids, the α-helix is destabilized, allowing the use of D-amino acid analogues in structure−activity studies to probe the relationship between conformational domains and bioactivity.[8] The extent of the destabilization of right-handed helices by a single D-amino acid substitution has been shown to be about 4 kJ mol^{-1} for alanine in a model peptide.[9,10] This is in good agreement with the theoretical values obtained by Hermans et al.[11] The effects of two D-amino acid substitutions in amphipathic peptides, peptide antibiotics, and neuropeptides have also been reported.[12−14] The all-D-analogues of helical all-L-peptides are expected to form left-handed helices, a feature which has been used in generating novel peptide ligands,[15] in designing heterochiral coiled coils,[16] and in constructing ambidextrous structures containing both right- and left-handed helical segments.[17] Alternating L- and D-residue peptides are used for the construction of cyclic peptides and nanotubes.[18,19]

Here, we describe a study of the effects of D-residue substitutions on the helical structure of unsolvated alanine-based peptides. The structures were probed using ion mobility mass spectrometry. The mobility of an ion (how quickly it travels through a buffer gas under the influence of a weak electric field) depends on its average collision cross-section. Ions with open or extended structures undergo more collisions with the buffer gas and travel more slowly than ions with compact structures.[20−22] Thus, peptides in a helical conformation travel more slowly (have a larger average collision cross-section) than peptides with compact globular conformations.[23−26] Previous studies have shown that alanine-based peptides with a lysine residue at the C-terminus form stable helices in the gas phase.[23,27,28] In these peptides, the protonated lysine side chain at the C-terminus interacts favorably with the helix macrodipole, stabilizing the helix. Recent studies have shown that the helical conformation of protonated $AcA_{15}K$ is stable up to 500 °C in the gas phase.[28] In this work, unsolvated $AcA_{15}K + H^+$ was used to study the helix destabilizing effect of D-residue substitution of L-residues. Our results show that the helix is disrupted when five or more residues are randomly replaced with D-residues. If the D-residues are placed in a block, the result is an ambidextrous structure with different helical segments with opposite chirality. An $AcA_{15}K + H^+$ peptide with all D-residues forms a left-handed helix in the gas phase.

Materials and Methods

The peptides used in this study were synthesized using *FastMoc* chemistry on an Applied Biosystems 433A peptide synthesizer. After synthesis, they were cleaved from the HMP (*p*-Hydroxymethylphenoxymethyl) polystyrene resin using a 95% trifluoroacetic acid (TFA) and 5% water v/v mixture, precipitated using cold diethyl ether, and lyophilized. The peptides were used without further purification. Solutions of 1 mg of peptide in a mixture of 1 mL of TFA and 0.1 of mL water were electrosprayed directly.

Mobility measurements were performed using a home-built ion mobility mass spectrometer which is described fully in a previous paper.[29] Electrosprayed peptide ions enter a differentially pumped region through a heated capillary (380−400 K) which helps to desolvate them and reduce the abundance of multimers. The ions are focused into the drift tube by electrostatic lenses. The drift tube temperature was varied from 173 to 400 K. After the ions exit the drift tube, they are passed through a quadrupole mass spectrometer and are then detected with a collision dynode and two stacked microchannel plates. The time taken by the ions to travel across the drift tube (the drift time) is determined using an electrostatic shutter to permit 100-μs packets of ions to enter the drift tube and recording their arrival time distribution at the detector using a multichannel scaler that is synchronized with the electrostatic shutter. Peaks in the drift time distributions are assigned by comparing their collision cross-sections (which are proportional to their drift

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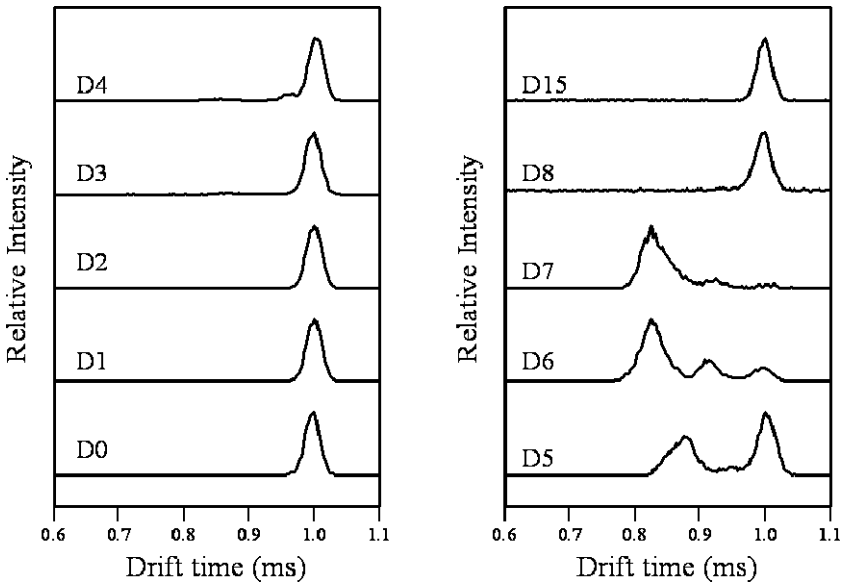

Figure 1. Drift time distribution of peptides **D0**−**D15** at 193 K.

TABLE 1: Percent Helix Observed at 193 K for Peptides D0−**D15**

sequence number	peptide sequence	number of D-residues	% helix observed at 193 K
D0	$AcA_{15}K$	0	100
D1	$AcA_7{}^DAA_7K$	1	100
D2	$AcA_6{}^DAA^DAA_6K$	2	100
D3	$AcA_5{}^DAA^DAA^DAA_5K$	3	100
D4	$AcA^DAA^DAA_7{}^DAA^DAAK$	4	93
D5	$AcA_3{}^DAA^DAA^DAA^DAA^DAA_3K$	5	50
D6	$AcA^DAA^DAA^DAA_3{}^DAA^DAAA^DAK$	6	15
D7	$AcA^DAA^DAA^DAA^DAA^DAA^DAA^DAK$	7	8
D8	$Ac^DA_8A_7K$	8	100
D15	$Ac^DA_{15}K$	15	100

times)[30] to average collision cross-sections calculated for conformations derived from molecular dynamics (MD) simulations.

MD simulations were performed using the *MACSIMUS* suite of programs[31] with the CHARMM 21.3 parameter set. Simulated annealing runs of 960-ps duration (using a schedule with a stepped linear temperature decrease) were started from either a fully extended or a fully helical conformation. Average collision cross-sections were calculated using the empirically corrected exact hard-spheres scattering model from 50 snapshots taken from the last 35 ps of the simulations.[32] If the structures sampled in the MD simulations match those present in the experiment, the measured and calculated cross-sections are expected to agree to within 2%. All MD simulations were terminated at room temperature, and the cross-section calculations were performed at room temperature.

Results

The peptides used in this study are listed in Table 1. Up to three peaks are observed in the drift time distributions recorded at low temperature (193 K) (see Figure 1). The peak at around 1.0 ms corresponds to a helical structure. The helix peak is the major peak observed for peptides **D0**−**D4**, **D8**, and **D15**. The peak at around 0.82 ms corresponds to globule, a compact random-looking three-dimensional structure. A significant amount of this conformation is observed for peptides **D5**−**D7**. These assignments will be discussed further below. The drift time distribution for peptide **D6** has three peaks: a helix, a globule, and another peak which has a cross-section between that of the helix and the globule. The percent helix at 193 K (obtained from the drift time distributions) shows that the helical content decreases to 50% in peptide **D5,** which has five D-residues and decreases to below 10% in peptide **D7**, which has seven D-residues. Peptides **D8** and **D15** are completely helical. **D8** has eight D-residues and **D15** has fifteen.

Above room temperature, all peptides except peptide **D5** show only a single peak in the drift time distributions. For peptides **D0**−**D4**, **D8**, and **D15**, the drift time distribution above room temperature shows only the helix peak. In peptides **D6** and **D7**, only the peak due to the globule is observed above room temperature. Peptide **D5** has two peaks up to a temperature of 392 K.

Representative low-energy structures obtained in the MD simulations that have calculated average cross-sections that correspond to the cross-sections deduced from the room-temperature drift time distributions are shown in Figure 2. Peptide **D0**, which has all L-residues, forms the right-handed helix shown in Figure 2a. The calculated cross-section for this structure is 304 Å^2, which matches the experimental cross-section of 305 Å^2. Peptide **D8**, which has a block of eight D-residues, forms an ambidextrous structure in the simulations, in which the helical screw sense reverses in the middle so that there are both right- and left-handed helical segments. The calculated cross-section for this structure is 301 Å^2, which is in good agreement with the measured value of 299 Å^2. Peptide **D15**, with fifteen D-residues, forms a left-handed helix in the simulations such as that shown in Figure 2c. The calculated cross-section for this structure is 300 Å^2, which matches the experimental value of 299 Å^2. The calculated cross-section for the distorted helix shown in Figure 2d (278 Å^2) matches the cross-section for the middle peak for peptide **D6** (277 Å^2). Figure 2e is an example of a low-energy globular structure. The calculated cross-section for this structure is 261 Å^2, which is slightly larger than cross-sections deduced from the peaks assigned to the globule for peptides **D5**−**D7** (**D5**, 258 Å^2; **D6**, 254 Å^2; **D7**, 254 Å^2). This illustrates a common problem: MD simulations have trouble finding compact low-energy globular conformations. Recently, it has been shown that a genetic algorithm can locate low-energy globular conformations that have cross-sections that closely match the experimental values,

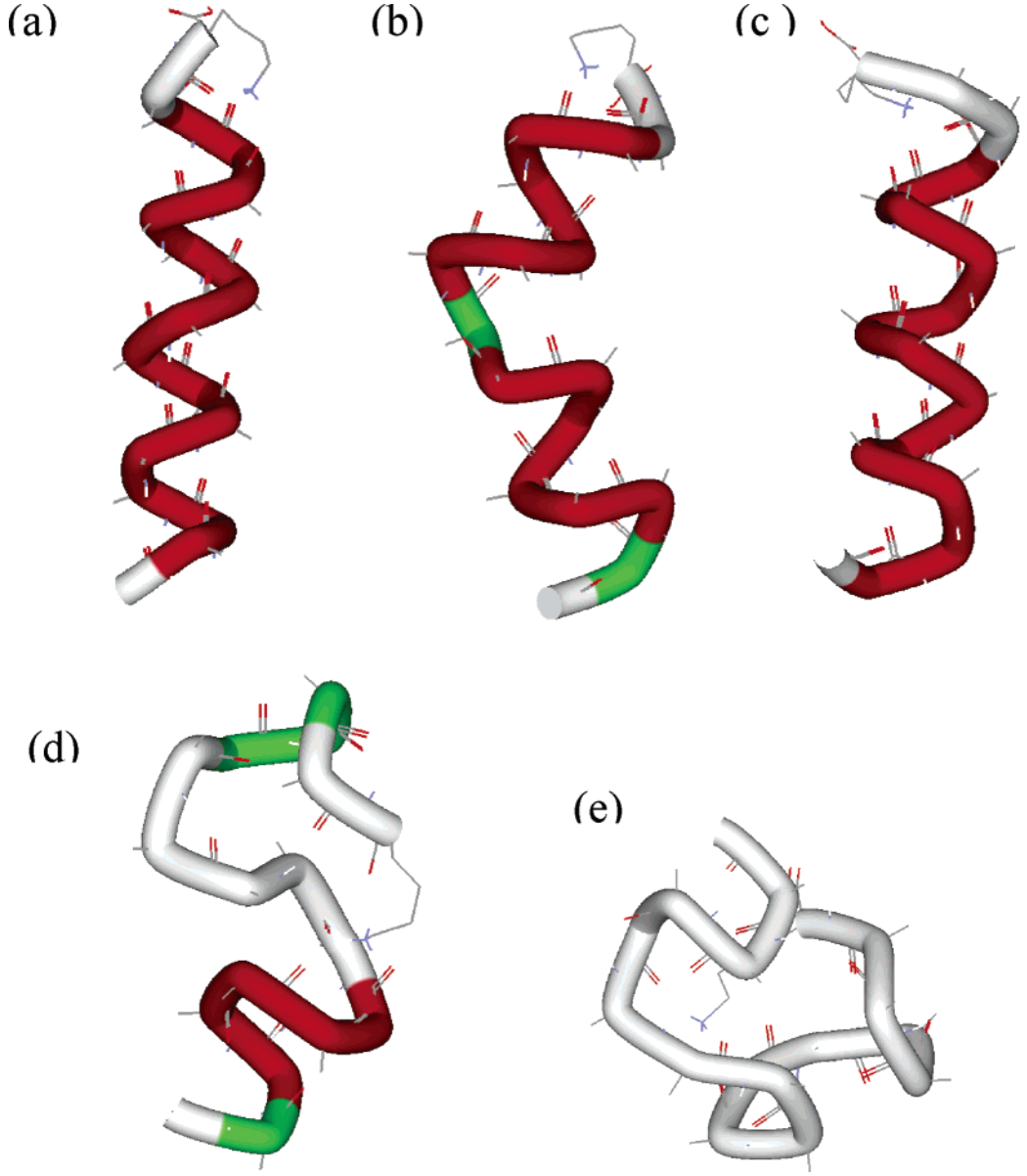

Figure 2. Representative low-energy structures obtained in the MD simulations of peptides **D0**, **D6**, **D8**, and **D15**. (a) Right-handed helix for peptide **D0**; (b) ambidextrous helix for peptide **D8**; (c) left-handed helix for peptide **D15**; (d) distorted helical structure observed in peptide **D6**; and (e) a representative globular structure.

though this requires the expenditure of substantial computational resources.[33] Because it is difficult to conceive of a conformation more compact than the globule, we did not pursue this issue further here.

Discussion

D-residues destabilize right-handed helices by unfavorable steric interactions between the side chain of the D-residue and the backbone. The β-carbon of the D-residue at position i has unfavorable steric interactions with the carbonyl oxygens of the i and $i - 1$ residues in the right-handed helical conformation. Also, the backbone angles available to a D-alanine in the right-handed α-helical portion of the Φ,Ψ map are more restricted and of higher energy than for L-alanine.[11] Our study shows that an unsolvated $AcA_{15}K + H^+$ helix can have as many as four D-residue substitutions without compromising the helical conformation. However, with the fifth substitution, a significant amount of the globular conformation is formed, because the helix is destabilized to the point where its energy is comparable to that of the globule (which is not expected to be sensitive to the D-residue composition). It has been reported that in the crystal structure of an eighteen-residue peptide, three D-residues could be accommodated without substantially disrupting the helix.[34] The number of D-residues required to destabilize a right-handed helix can vary depending on the environment and on the nature of the amino acid residues in the peptide. Amino acids with bulky and branched side chains are expected to destabilize the helix more readily than amino acids with smaller side chains.[35] For the unsolvated peptides, the helical conformation is stabilized by electrostatic interactions involving the helix macrodipole. The distortions caused by D/L-substitutions are expected to diminish the electrostatic interactions that stabilize the helix by partially disrupting the helix macrodipole.

Peptide **D8** forms a stable helix even though it has eight D-residues. This is because the D-residues are placed in a block. The helix content decreases in peptides having five or more D-residues at random positions, while a block of eight D-residues leads to a conformation with a cross-section that is similar to

that of peptide **D0**, which is a right-handed helix. The MD simulations indicate that the **D8** helix must be ambidextrous with two helical segments of the opposite screw sense, as shown in Figure 2b.

Peptide **D15**, with all D-residues, forms a left-handed helix with a similar cross-section to that of peptide **D0**. The cross-sections of the right-handed and left-handed helices are the same. Hence, it is not possible to determine the screw sense of the helix on the basis of the mobility measurements alone. However, the observation that peptide **D15** forms a stable helix, while the presence of five or more D-residues destabilize a right-handed helix, indicates that **D15** must be a helix with the opposite screw sense. Solution studies have shown that the all-D-residue peptides form left-handed helices.[36]

Conclusions

D-residue substitutions in unsolvated alanine-based peptides are helix destabilizing. Although the helical conformation of the protonated $AcA_{15}K$ peptide is stable up to 500 °C in the gas phase, randomly replacing five residues with their D-analogues destabilizes the helix to the point where the globule becomes energetically competitive. With more than five random substitutions, the globule becomes the dominant conformation. Substituting a block of residues leads to an ambidextrous helix with both right- and left-handed components. The all-D-residue peptide forms a left- handed helix. The behavior observed for the unsolvated peptides is quite similar to that found in solution and in crystals (where a handful of D-residue substitutions disrupt the helical state). The disruption is primarily caused by unfavorable steric interactions between the side chain of the D-residue and the backbone. In the unsolvated peptides, where the helical conformation is stabilized by electrostatic interactions involving the helix macrodipole, the distortions caused by D/L-substitutions are expected to also diminish the electrostatic interactions that stabilize the helix by partially disrupting the helix macrodipole. The extent of the destabilization of right-handed helices by a single D-amino acid substitution could be quanitified using ion mobility studies in a suitable model system with a mixed population of helix and globule.

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References and Notes

(1) Pauling, L.; Corey, R. B.; Branson, H. R. *Proc. Natl. Acad. Sci. U.S.A.* **1951**, *37*, 205−211.
(2) Kreil, G. *Annu. Rev. Biochem.* **1997**, *66*, 337−345.
(3) Richter, K.; Egger, R.; Negri, L.; Corsi, R.; Severino, C.; Kreil, G. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 4836−4839.
(4) Kreil, G. *Science* **1994**, *266*, 996−997.
(5) Bessalle, R.; Kapitkovsky, A.; Gorea, A.; Shalit, I.; Fridkin, M. *FEBS Lett.* **1990**, *274*, 151−155.
(6) Hong, S. Y.; Oh, J. E.; Lee, K.-H. *Biochem. Pharmacol.* **1999**, *58*, 1775−1780.
(7) Papo, N.; Shai, Y. *Biochemistry* **2003**, *42*, 9346−9354.
(8) Pouny, Y.; Shai, Y. *Biochemistry* **1992**, *31*, 9482−9490.
(9) Fairman, R.; Anthony-Cahill, S. J.; DeGrado, W. F. *J. Am. Chem. Soc.* **1992**, *114*, 5458−5459.
(10) Chen, Y.; Mant, C. T.; Hodges, R. S. *J. Pept. Res.* **2002**, *59*, 18−33.
(11) Hermans, J.; Anderson, A. G.; Yun, R. H. *Biochemistry* **1992**, *31*, 5646−53.
(12) Rothemund, S.; Beyermann, M.; Krause, G.; Bienert, M.; Hodges, R. S. Sykes, B. D.; Sönnichsen, F. D. *Biochemistry* **1995**, *34*, 12954−12962.
(13) Wieprecht, T.; Dathe, M.; Schümann, M.; Krause, E.; Beyermann, M.; Bienert, M. *Biochemistry* **1996**, *35*, 10844−10853.
(14) Krause, E.; Beyermann, M.; Dathe, M.; Rothemund, S.; Bienert, M. *Anal. Chem.* **1995**, *67*, 252−258.
(15) Van Regenmortel, M. H. V.; Muller, S. *Curr. Opin. Biotechnol.* **1998**, *9*, 377−382.
(16) Sia, S. K.; Kim, P. S *Biochemistry* **2001**, *40*, 8981−8989.
(17) Banerjee, A.; Raghothama, S.; Karle, I. L.; Balaram, P. *Biopolymers* **1996**, *39*, 279−285.
(18) Ghadiri, M. R.; Granja, J. R.; Milligan, R. A.; McRee, D. E.; Khazanovich, N. *Nature (London)* **1993**, *366*, 324−327.
(19) Rosenthal-Aizman, K.; Svensson, G.; Undén, A. *J. Am. Chem. Soc.* **2004**, *126*, 3372−3373.
(20) Hagen, D. F. *Anal. Chem.* **1979**, *51*, 870−874.
(21) Von Helden, G.; Hsu, M. T.; Kemper, P. R.; Bowers, M. T. *J. Chem. Phys.* **1991**, *95*, 3835−3837.
(22) Clemmer, D. E.; Jarrold, M. F. *J. Mass Spectrom.* **1997**, *32*, 577−592.
(23) Hudgins, R. R.; Ratner, M. A.; Jarrold, M. F. *J. Am. Chem. Soc.* **1998**, *120*, 12974−12975.
(24) Hudgins, R. R.; Jarrold, M. F. *J. Phys. Chem. B* **2000**, *104*, 2154−2158.
(25) Hartings, M. R.; Kinnear, B. S.; Jarrold, M. F. *J. Am. Chem. Soc.*, **2003**, *125*, 3941−3947.
(26) Counterman, A. E.; Clemmer, D. E. *J. Am. Chem. Soc.* **2001**, *123*, 1490−1498.
(27) Hudgins, R. R.; Jarrold, M. F. *J. Am. Chem. Soc.* **1999**, *121*, 3494−3501.
(28) Kohtani, M.; Jones, T. C.; Schneider, J. E.; Jarrold, M. F. *J. Am. Chem. Soc.* **2004**, *126*, 7420−7421.
(29) Kinnear, B. S.; Hartings, M. R.; Jarrold, M. F. *J. Am. Chem. Soc.* **2001**, *123*, 5660−5667.
(30) Mason, E. A.; McDaniel, E. W. *Transport Properties of Ions in Gases*; Wiley: New York, **1988**.
(31) Kolafa, J. *MACSIMUS*; http://www.icpf.cas.cz/jiri/macsimus/default.htm.
(32) Kinnear, B. S.; Kaleta, D. T.; Kohtani, M.; Hudgins, R. R.; Jarrold, M. F. *J. Am. Chem. Soc.* **2000**, *122*, 9243−9256.
(33) Damsbo, M.; Kinnear, B. S.; Hartings, M. R.; Ruhoff, P. T.; Jarrold, M. F.; Ratner, M. A. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 7215−7222.
(34) Karle, I. L.; Gopi, H. N.; Balaram, P. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 13946−13951.
(35) Krause, E.; Bienert, M.; Schmieder, P.; Wenschuh, H. *J. Am. Chem. Soc.* **2000**, *122*, 4865−4870.
(36) Das, C.; Berezovska, O.; Diehl, T. S.; Genet, C.; Buldyrev, I.; Tsai, J.; Hyman, B. T.; Wolfe, M. S. *J. Am. Chem. Soc.* **2003**, *125*, 11794−11795.