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Peptide Pinwheels

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Ion mobility measurements and molecular dynamics (MD) simulations suggest that the unsolvated trimers produced by electrospraying a mixture of Ac-$(GA)_7$K and Ac-A$(GA)_7$K (Ac = acetyl, G = glycine, A = alanine, and K = lysine) have unusual pinwheel geometries consisting of three helices tethered together by the protonated lysine side chain from one peptide interacting with the C-terminus of a neighboring helix, see Figure 1a. In this geometry, the charged groups are localized close to the axis of the pinwheel—an arrangement that at first glance seems to be both unfavorable and unlikely. However, MD simulations show this arrangement to be advantageous because it leads to a cooperative electrostatic stabilization through the interaction of the combined charge with all the helix dipoles. The dimer adopts a related V-shaped arrangement of helices which is also cooperatively stabilized, see Figure 1b.

Determining how secondary structure associates into the tertiary structure domains of proteins is central to understanding the connection between sequence and structure.[1] Most studies have been performed in solution, where it is often difficult to unravel the complex interplay between intramolecular and solution interactions.[2] Furthermore, aqueous solution is not the only biologically important environment; membrane proteins make up to 30% of the proteins encoded by genomes,[3] and the hydrophobic interior of a lipid bilayer is strikingly different from an aqueous environment. Studies of unsolvated peptides and proteins provide information on their intrinsic properties which provides a basis for understanding their behavior throughout the biological milieu.[4] In the work described here we examine the unsolvated aggregates of helix-forming peptides. The interactions between helical peptides have been investigated as models for understanding the formation of ion channels,[5−7] which are an important structure in cell membranes and in the action of antipathogenic drugs.[8]

The aggregates studied here were generated by electrospray.[9] Electrospray is a sufficiently gentle ionization technique that weakly bound noncovalent complexes can often be transferred directly into the gas phase,[10,11] and the observation of peptide aggregates is fairly common.[12−14] The conformations of the complexes were probed by using ion mobility measurements. The mobility of a gas phase ion (how rapidly it moves through a buffer gas under the influence of a weak electric field) depends on its average collision cross section which in turn depends on its structure. Ions with compact, folded structures travel more rapidly than ions with more open conformations. The ion mobility apparatus used in these studies has been described previously.[15]

Figure 2a shows a typical mass spectrum obtained by electrospraying a 1:1 mixture of Ac-$(GA)_7$K and Ac-A$(GA)_7$K peptides. Abundant peaks due to the mixed dimer (Ac-$(GA)_7$KAAc-A$(GA)_7$K+$2H^+$) and mixed trimers (2Ac-$(GA)_7$KAAc-A$(GA)_7$K+$3H^+$ and Ac-$(GA)_7$KA2Ac-A$(GA)_7$K+$3H^+$) are evident between the Ac-$(GA)_7$K+H^+ and Ac-A$(GA)_7$K+H^+ peaks. Note that the peaks labeled Ac-$(GA)_7$K+H^+ and Ac-A$(GA)_7$K+H^+ consist of monomers and homomultimers (dimers and trimers with the same mass-to-charge ratios as the monomers). Ac-$(GA)_7$K+H^+ and Ac-A$(GA)_7$K+H^+ are designed to be marginally stable helices in the gas phase.[16] The protonated lysine side-chain stabilizes the helical state through favorable interactions with the helix dipole and by

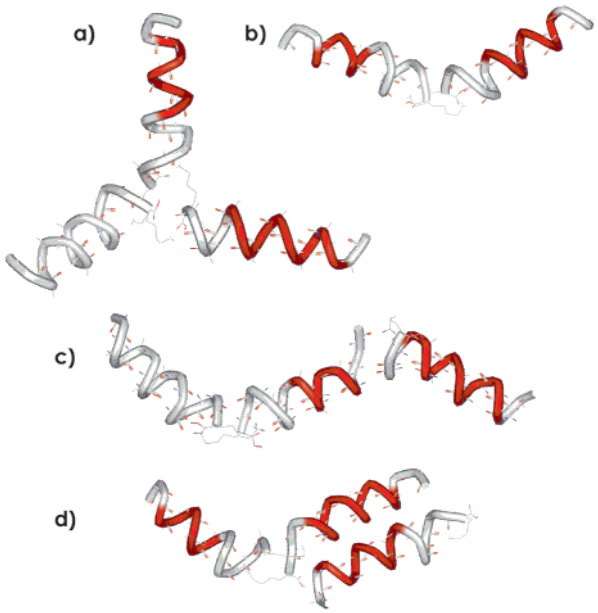

Figure 1. Conformations for dimer and trimers obtained from MD simulations: (a) pinwheel geometry for low mass trimer; (b) V-shaped dimer; (c) V-shaped dimer with collinear helix; and (d) V-shaped dimer with antiparallel helix. The images were produced using the WebLab viewer (Molecular Simulations Inc., San Diego, CA). The red regions are α-helical.

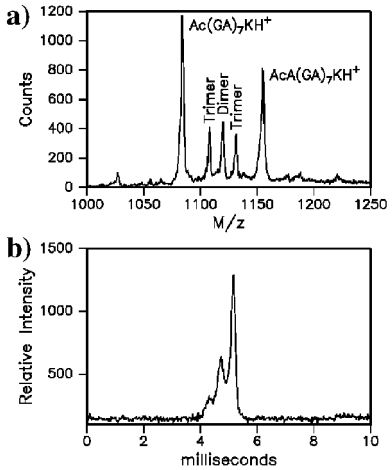

Figure 2. (a) Electrospray mass spectrum recorded for a 1:1 mixture of Ac-$(GA)_7$K and Ac-A$(GA)_7$K in 90% trifluoroacetic acid/10% water. (b) Drift time distributions recorded for the low mass trimer (2Ac-$(GA)_7$KAAc-A$(GA)_7$K+$3H^+$). The distribution for the high mass trimer is almost identical.

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capping (hydrogen bonds to the dangling carbonyl groups at the C-terminus).[17,18] The glycine residues, on the other hand, destabilize the helical state. Ion mobility measurements indicate that the monomers are predominantly helical, but with a small component (~15%) assigned to a globule. Figure 2b shows the drift time distribution (DTD) measured for the low mass trimer (2Ac-$(GA)_7$KAAc-A$(GA)_7$K+3H$^+$). It shows three peaks: a narrow peak at long drift times, and two broader and smaller peaks at shorter drift times. The DTD for the high mass trimer shows similar features, but with slightly longer drift times.

Information about the nature of the conformations present in the DTDs is obtained by comparing the cross sections deduced from the meansurements to orientationally averaged values calculated for geometries obtained from molecular dynamics (MD) simulations. The MD simulations were performed with the MACSIMUS suite of programs[19] using CHARMM potentials.[20] Cross sections were calculated by using an empirical correction to the exact hard spheres scattering model,[21] averaging over 50 snapshots taken from the final 35 ps of the simulation. If the conformation is correct the calculated cross section is expected to be within 2% of the measured value. A large number of simulations were performed, both fixed temperature and simulated annealing.[22,23] Starting conformations for the simulations were made by manipulating monomers (ideal α-helices, low energy globules from monomer simulations, or linear chains) by hand within a graphical environment. A wide range of different starting conformations were employed, including various planar and nonplanar helical aggregates, triangular head-to-toe helices, and extended coaxial arrangements of helices. All possible orientations of the helix dipoles were considered. For helices there are two distinct arrangements for the lysine side chains. The protonated lysine may loop around and interact with the C-terminus of the same peptide (which we refer to as unexchanged) or it may interact with the C-terminus of another helical peptide (exchanged). In some cases, peptides that started as unexchanged switched to exchanged, but we never observed the reverse process.

While many conformations are possible for the trimer, examination of the MD simulations shows that there are only a few categories of low-energy conformations. The cross sections for the pinwheel geometry shown in Figure 1a match the measured values for the most abundant feature in the trimer DTDs. In this geometry the three helices radiate out like the spokes of a wheel and they are linked by the protonated lysine side chains associating with the C-termini of neighboring helices (the "exchanged" arrangement for all peptides). There is a cooperative electrostatic stabilization of all the helices in this geometry: all the helix dipoles point toward all the charges, which compensates for the repulsive interactions between the charges which are localized near the center. Unfavorable interactions between the helix dipoles are also minimized in this arrangement. Elevated temperature MD simulations were performed to test the stability of the pinwheel, it survived 1 ns MD simulations at 600 K without dissociating. The activation barrier for uncoupling the protonated lysine side chains from the C-termini of adjacent peptides probably helps to maintain the geometry and resist dissociation.

Another common motif found for the trimer consists of a V-shaped dimer complexed with another helix in various orientations. The V-shaped dimer with exchanged lysines shown in Figure 1b is the lowest energy structure found for the Ac-$(GA)_7$KAAc-A$(GA)_7$K+2H$^+$ dimer and its cross section matches that for the most abundant feature in the dimer DTD. A similar dimer has been found for polyalanine based peptides.[14] Trimer conformations that incorporate the V-shaped dimer are shown in Figure 1c,d. Figure 1c is a roughly collinear arrangement that is stabilized by favorable alignment of the helix dipoles of the second and third helices. It is only slightly less stable than the pinwheel and has a cross section that matches the dominant feature in the trimer DTDs. Elevated temperature MD simulations were performed to test the stability of the collinear arrangement. It survived 1 ns simulations at 320 K, but dissociated, losing the third helix, at 340 K. Because of the low activation barrier to dissociation, the collinear arrangement is probably not an important contributor to the measured DTDs.

Figure 1d shows a V-shaped dimer with a third helix oriented in an antiparallel, coiled-coil arrangement. The energies for this conformation are also comparable to the pinwheels. However, the calculated cross sections match the second most abundant feature in the trimer DTDs. Slightly different arrangements of the V-shaped dimer and antiparallel helix lead to a fairly broad range of cross sections that may explain why the second most abundant feature in the trimer DTDs is quite broad (see Figure 2). The antiparallel helix arrangement survived 1 ns MD simulations at 400 K but dissociated in 500 K simulations. The cross sections for the least abundant feature in the trimer DTDs are matched by conformations with three side-by-side helices and by conformations that contain mixtures of helices and globules.

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References

(1) Branden, C.; Tooze, J. *Introduction to Protein Structure*, 2nd ed.; Garland: New York, 1999.
(2) Dill, K. A. *Biochemistry* **1990**, *29*, 7133−7155.
(3) Wallin, E.; von Heijne, G *Protein Sci.* **1998**, *7*, 1029−1038.
(4) See, for example: Suckau, D.; Shi, Y.; Beu, S. C.; Senko, M. W.; Quinn, J. P.; Wampler, F. M.; McLafferty, F. W. *Proc. Natl. Acad. Sci.* **1993**, *90*, 790−793. Campbell, S.; Rodgers, M. T.; Marzluff, E. M.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1995**, *117*, 12840−12854. Schnier, P. D.; Price, W. D.; Jockusch, R. A.; Williams, E. R. *J. Am. Chem. Soc.* **1996**, *118*, 7178−7189. Kaltashov, I. A.; Fenselau, C. *Proteins: Struct. Func. Genet.* **1997**, *27*, 165−170. Counterman, A. E.; Clemmer, D. E. *J. Am. Chem. Soc.*, **2001**, *123*, 1490−1498. Wyttenbach, T.; Bushnell, J. E.; Bowers, M. T. *J. Am. Chem. Soc.* **1998**, *120*, 5098−5103. Arteca, G. A.; Velázquez, I.; Reimann, C. T.; Tapia, O. *Phys. Rev. E* **1999**, *59*, 5981−5986. Schaaff, T. G.; Stephenson, J. L.; McLuckey, S. L. *J. Am. Chem. Soc.* **1999**, *121*, 8907−8919. Kinnear, B. S.; Jarrold, M. F. *J. Am. Chem. Soc.* **2001**, *123*, 7907−7908.
(5) Sansom, M. S. P. *Prog. Biophys. Mol. Biol.* **1991**, *55*, 139−236.
(6) Kerr, I. D.; Sankararamakrishnan, R.; Smart, O. S.; Sansom, M. S. P *Biophys. J.* **1994**, *67*, 1501−1535.
(7) Randa, H. S.; Forrest, L. R.; Voth, G. A.; Sansom, M. S. P *Biophys. J.* **1999**, *77*, 2400−2410.
(8) Pandey, R. C.; Meng, H.; Cook, J. C., Jr.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1977**, *99*, 5203−5205.
(9) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Science* **1989**, *246*, 64−71.
(10) Light-Wahl, K. J.; Schwartz, B. L.; Smith, R. D. *J. Am. Chem. Soc.* **1994**, *116*, 5271−5278.
(11) Loo, J. *Int. J. Mass Spectrom.* **2000**, *200*, 175−186. Smith, R. D. *Int. J. Mass Spectrom.* **2000**, *200*, 509−544.
(12) Counterman, A. E.; Valentine, S. J.; Srebalus, C. A.; Henderson, S. C.; Hoaglund, C. S.; Clemmer, D. E. *J. Am. Soc. Mass Spectrom.* **1998**, *9*, 746−759.
(13) Lee, S.-W.; Beauchamp, J. L *J. Am. Soc. Mass Spectrom.* **1999**, *10*, 347−351.
(14) Hudgins, R. R.; Jarrold, M. F. *J. Am. Chem. Soc.* **1999**, *121*, 3494−3501.
(15) Kinnear, B. S.; Hartings, M. R.; Jarrold, M. F. *J. Am. Chem. Soc.* **2001**, *123*, 5660−5667.
(16) Hudgins, R. R.; Ratner, M. A.; Jarrold, M. F. *J. Am. Chem. Soc.* **1998**, *120*, 12974−12975.
(17) Presa, L. G.; Rose, G. D. *Science* **1988**, *240*, 1632−1641.
(18) Seale, J. W.; Srinivasan, R.; Rose, G. D. *Protein Sci.* **1994**, *3*, 1741−1745.
(19) http://www.icpf.cas.cz/jiri/macsimus/default.htm.
(20) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187−217.
(21) Kinnear, B. S.; Kaleta, D. T.; Kohtani, M.; Hudgins, R. R.; Jarrold, M. F. *J. Am. Chem. Soc.* **2000**, *122*, 9243−9256.
(22) Kirkpatrick, S.; Gelatt, C. D., Jr.; Vecchi, M. P. *Science* **1983**, *220*, 671−680.
(23) Kaleta, D. T.; Jarrold, M. F. *J. Phys. Chem. B* **2001**, *105*, 4436−4440.

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