J|A|C|S COMMUNICATIONS

Published on Web 09/02/2010

Oligo(4-aminopiperidine-4-carboxylic acid): An Unusual Basic Oligopeptide with an Acid-Induced Helical Conformation

Joon-il Cho,[†] Masahiro Tanaka,[†] Sota Sato,[‡] Kazushi Kinbara,[†] and Takuzo Aida[†,*]

Department of Chemistry and Biotechnology and Department of Applied Chemistry, School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan

Received July 10, 2010; E-mail: aida@macro.t.u-tokyo.ac.jp

Abstract: In sharp contrast with helical polypeptides carrying basic side chains, Api_8, a basic oligopeptide containing the non-natural achiral amino acid 4-aminopiperidine-4-carboxylic acid (Api), adopts a helical conformation only in acidic media. Alkaline titration of a protonated Api_8 oligomer appended with a leucine derivative at its N-terminus showed that disruption of its helical conformation occurs in a pH range of 7−10. NMR studies indicated that the piperidine groups in Api_8, when nonprotonated, possibly interact with the proximal amide protons in the peptide backbone and hamper the formation of the H-bonding network responsible for the helical conformation. The helical structure is induced not only by protonation but also by acylation of the piperidine groups.

Natural polypeptides containing basic amino acids such as lysine and arginine play crucial roles in biological events.[1] Conformational studies of their synthetic analogues have indicated that protonation of the basic side chains of these amino acid residues results in a helix-to-coil transition as a result of disruption of the H-bonding network along the peptide backbone by an electrostatic repulsion.[2] Such a protonation-induced conformational change has inspired synthetic chemists to design a variety of pH-responsive nonpeptidic analogues appended with basic functional groups.[3] However, with only a few exceptions,[3c−e] all previously reported pH-responsive helical motifs having basic functional groups, including foldamers,[3a] adopt a helical conformation only in nonacidic media.[3] Here we report that oligo(4-aminopiperidine-4-carboxylic acid) (Api_n) is the first basic oligopeptide that adopts a stable helical conformation only in acidic media.

Api is a non-natural amino acid bearing a piperidine group and has no chiral center. By analogy to oligopeptides containing other α,α-disubstituted achiral amino acids,[4] Api_n is presumed to adopt a helical conformation as a result of not only intramolecular H-bonding interactions but also steric repulsion among the α-substituents. However, homotropic Api_n has never been reported; only Api-containing copolypeptides[5] are known. Thus, we first established an iterative synthetic method for Api_n starting from Api_2.[6] For a conformational study of Api_n using circular dichroism (CD) spectroscopy, Api_8 carrying *N*-acetylleucine (L- or D-Leu^{Ac}) at its N-terminus [$Leu^{Ac}(Api_8)OBn$; Figure 1] was synthesized[6] with an expectation that Leu as a chiral auxiliary could allow Api_8 to adopt a prevailing one-handed helical conformation. However, to our surprise, $Leu^{Ac}(Api_8)OBn$ did not show any CD sign typical of helical structures. We later found that protonation of the piperidine groups is quite essential for $Leu^{Ac}(Api_8)OBn$ to adopt a helical conformation (Figure 1).

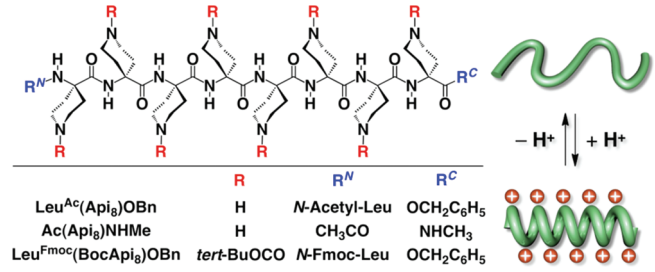

Figure 1. Schematic representations of Api octapeptides and their transition to a helical conformation induced by H^+.

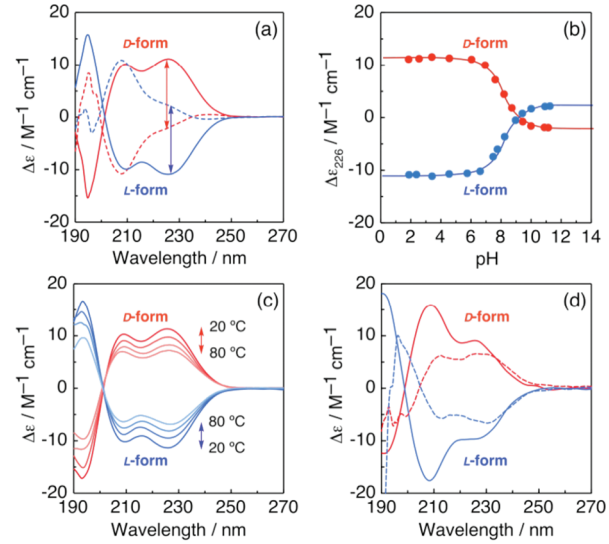

Figure 2. (a) CD spectra at 20 °C for $Leu^{Ac}(Api_8)OBn$ (0.03 mM) at pH 4 (solid curves) and pH 10 (broken curves). (b) Plots of Δε at 226 nm for $Leu^{Ac}(Api_8)OBn$ (0.03 mM) vs pH at 20 °C. (c) CD spectra at 20−80 °C for $Leu^{Ac}(Api_8)OBn$ (0.03 mM) at pH 4. (d) CD spectra at 20 °C for $Leu^{Fmoc}(BocApi_8)OBn$ (0.15 mM) in MeCN (solid curves) and MeOH (broken curves).

As shown in Figure 2a, an acidic aqueous solution (pH 4) of L-$Leu^{Ac}(Api_8)OBn$ (0.03 mM) at 20 °C displayed negative-signed Cotton effects at 210 ($\pi-\pi^*$) and 226 nm ($n-\pi^*$). As expected, the CD spectrum of D-$Leu^{Ac}(Api_8)OBn$ under conditions identical to the above was the mirror image of that observed for the L-form. Since the ratio of the Δε values at 210 and 226 nm ($\Delta\varepsilon_{226}/\Delta\varepsilon_{210}$) was almost unity, the octameric Api moiety (Api_8) in $Leu^{Ac}(Api_8)OBn$ most likely adopts an α-helical conformation.[7] The observed CD spectral profile remained virtually unchanged upon a decrease in the pH (e.g., to 2). In sharp contrast, under basic conditions with pH ≥ 10, $Leu^{Ac}(Api_8)OBn$ showed a CD spectral feature (Figure 2a) with a shape similar to that of monomeric $Leu^{Ac}OBn$,[6] indicating that Api_8 without protonation adopts a

[†] Department of Chemistry and Biotechnology.
[‡] Department of Applied Chemistry.

10.1021/ja106118w

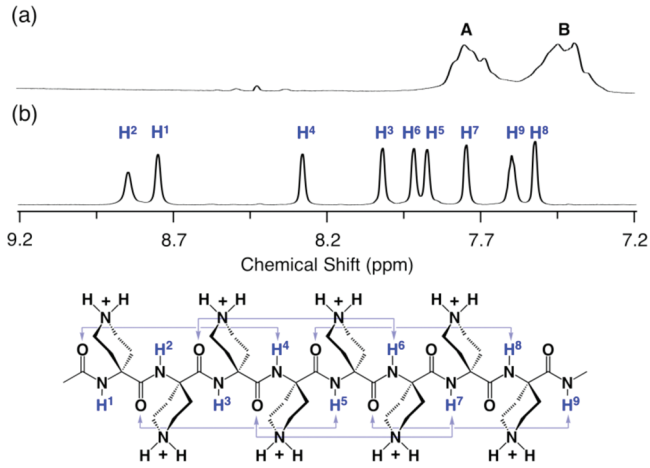

Figure 3. ^{1}H NMR spectra at 20 °C for Ac(Api$_8$)NHMe (5 mM) in water at (a) pH 9 and (b) pH 6. The sample tube included an inner sealed tube containing a D_2O solution of 4,4-dimethyl-4-silapentane-1-sulfonic acid sodium salt (DSS, 15 mM) as an internal standard. Arrows indicate H-bonded pairs.

nonhelical conformation. Alkaline titration of an acidic (pH 2) aqueous solution of Leu^{Ac}(Api$_8$)OBn resulted in an abrupt CD intensity change at pH 7−10 (Figure 2b). Although the pH value at the inflection point of this transition is less than the pK_a value of piperidine (11.1),[8] we conclude that protonation of the piperidine units allows Leu^{Ac}(Api$_8$)OBn to adopt an α-helical conformation. The helical structure is thermally stable, as the CD spectral profile of Leu^{Ac}(Api$_8$)OBn at pH 4 still maintained the characteristics of the α-helix even upon heating to 80 °C and recovered the original intensity (64 → 100%) completely upon cooling to 20 °C (Figure 2c). We also found the presence of a critical chain length for protonated Api$_n$ to adopt a stable helical conformation. A shorter-chain homologue such as Leu^{Ac}(Api$_4$)OBn is likely a critical oligomer, which at pH 2 displayed only a small CD spectral feature of the helical conformation.[6] The pH dependence of the conformational stability of α-helical Api$_8$ observed here is contrary to those reported for ordinary polypeptides bearing basic functional groups.[2] In relation to this interesting contrast, we found that Api$_8$ adopts a helical conformation when an electron-withdrawing group such as *tert*-butoxycarbonyl (Boc) is attached to the piperidine nitrogen atoms to decrease their basicity. For example, Leu^{Fmoc}(BocApi$_8$)OBn (Figure 1) in MeCN and MeOH at 20 °C displayed a CD spectral feature typical of helical conformations (Figure 2d).[9]

To elucidate a possible role of the piperidine groups in the conformational characteristics of Api$_8$, we measured ^{1}H NMR spectra of Ac(Api$_8$)NHMe (Figure 1) in water at 20 °C (Figure 3). Under acidic conditions (pH 6), the amide NH region (Figure 3b) displayed nine well-resolved signals (H^1−H^9). In an ^{1}H NMR saturation-transfer experiment upon selective irradiation of the water signal (4.6 ppm), amide NH signals H^4−H^9 decreased in intensity by 40−50%, whereas signals H^1−H^3 displayed more significant intensity decreases (70−90%).[6] The observed intensity changes are caused by the amide−water proton exchange. On the basis of the exchange rates, as evaluated by the saturation-transfer method,[6] the amide NHs in the former set (H^4−H^9) are likely involved in the H-bonding network, but the remaining three (H^1−H^3) are free. Together with ^{1}H−^{1}H correlations evaluated by 2D rotational Overhauser effect spectroscopy (ROESY),[6] all of the amide NH signals in Figure 3b were reasonably assigned to the α-helical conformation of protonated Ac(Api$_8$)NHMe. In sharp contrast, under basic conditions (pH 9), the amide NH region exhibited only two

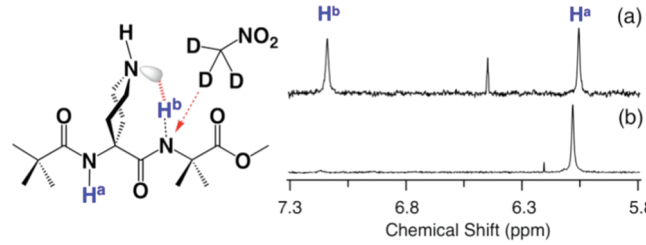

Figure 4. ^{1}H NMR spectra of ^{tert}Bu(Api)(Aib)OMe (5 mM) in (a) CH_3NO_2 and (b) CD_3NO_2 at 20 °C.[6]

broad peaks (Figure 3a), both of which became very weak upon selective irradiation of the water signal.[6] Hence, we conclude that Api$_8$ under nonacidic conditions is devoid of H-bonds along the peptide backbone.

By using ^{tert}Bu(Api)(Aib)OMe (Aib = 2-aminoisobutyric acid) (Figure 4) as a model compound for the repeating Api units, we found that the nonprotonated piperidine groups in Api$_8$ possibly hamper the H-bonding interactions of the amide units in the peptide backbone. ^{tert}Bu(Api)(Aib)OMe bears two amide NH groups that are located in similar steric environments but have different geometries with respect to the piperidine nitrogen atom. Interestingly, when CD_3NO_2 (pK_a = 10.2)[10] was used as the solvent, the ^{1}H NMR spectrum of ^{tert}Bu(Api)(Aib)OMe hardly showed the amide NH signal due to H^b as a result of H−D exchange, while the signal due to H^a remained intact (Figure 4).[11] In contrast, when the piperidine group of ^{tert}Bu(Api)(Aib)OMe was protonated, both H^a and H^b were detected.[6] The same was true for ^{tert}Bu(BocApi)(Aib)OMe, a Boc-protected version of ^{tert}Bu(Api)(Aib)OMe, in CD_3NO_2.[6] Since in either case the intrinsic acidities of H^a and H^b are likely comparable to one another, the above results clearly indicate that the piperidine nitrogen atom in ^{tert}Bu(Api)(Aib)OMe, when nonprotonated, interacts with amide N−H^b regioselectively and activates it for proton exchange. The observed regioselectivity is likely due to a restricted conformation of the piperidine group. Such an interaction could also occur in Api$_8$, causing its H-bonding network that is responsible for the helical conformation to be disrupted in nonacidic media.

In conclusion, we have demonstrated that oligomeric 4-aminopiperidine-4-carboxylic acid (Api$_n$) is the first basic peptide that adopts a helical conformation only in acidic media. When the piperidine groups of Api$_n$ are nonprotonated, they possibly interact with the proximal amide NH protons in the peptide backbone and hamper the formation of the H-bonding network responsible for the helical conformation. Utilization of Api$_n$ as a building block certainly provides many new possibilities for designing pH-responsive functional peptides and related chemistry.

Acknowledgment. We thank Professor Makoto Fujita for his generous support in NMR measurements.

Supporting Information Available: Details of syntheses, characterization data, and CD and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Martin, C.; Zhang, Y. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 838–849, and references therein. (b) Fawell, S.; Seery, J.; Daikh, Y.; Moore, C.; Chen, L. L.; Pepinsky, B.; Barsoum, J. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 664–668.
(2) (a) Greenfield, N. J.; Fasman, G. D. *Biochemistry* **1969**, *8*, 4018–4116. (b) Tseng, Y.-W.; Yang, J. T. *Biopolymers* **1977**, *16*, 921–935. (c) Hayakawa, T.; Kondo, Y.; Yamamoto, H. *Bull. Chem. Soc. Jpn.* **1969**, *42*, 1937–1941.
(3) (a) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893–4012. (b) Kolomiets, E.; Berl, V.; Odriozola, I.; Stadler, A.; Kyritsakas, N.; Lehn, J. M. *Chem. Commun.* **2003**, 2868–2869.

(c) Dolain, C.; Maurizot, V.; Huc, I. *Angew. Chem., Int. Ed.* **2003**, *42*, 2738–2740. (d) Majidi, M. R.; Kane-Maguire, L. A. P.; Wallace, G. G. *Polymer* **1995**, *36*, 3597–3599. (e) Yashima, E.; Maeda, Y.; Matsushima, T.; Okamoto, Y. *Chirality* **1997**, *9*, 593–600. (f) Okamoto, I.; Nabeta, M.; Hayakawa, Y.; Morita, N.; Takeya, T.; Masu, H.; Azumaya, I.; Tamura, O. *J. Am. Chem. Soc.* **2007**, *129*, 1892–1893. (g) Sebastian, H.; Hecht, S. *Macromolecules* **2010**, *43*, 242–248.

(4) (a) Venkatraman, J.; Shankaramma, S. C.; Balaram, P. *Chem. Rev.* **2001**, *101*, 3131–3152. (b) Marshall, G. R.; Hodgkin, E. E.; Langs, D. A.; Smith, G. D.; Zabrocki, J.; Leplawy, M. T. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 487–491. (c) Toniolo, C.; Benedetti, E. *Macromolecules* **1991**, *24*, 4004–4009. (d) Guo, Y. M.; Oike, H.; Aida, T. *J. Am. Chem. Soc.* **2004**, *126*, 716–717. (e) Guo, Y. M.; Oike, H.; Saeki, N.; Aida, T. *Angew. Chem., Int. Ed.* **2004**, *43*, 4915–4918.

(5) (a) Yokum, T. S.; Gauthier, T. J.; Hammer, R. P.; McLaughlin, M. L. *J. Am. Chem. Soc.* **1997**, *119*, 1167–1168. (b) Yokum, T. S.; Bursavich, M. G.; Gauthier, T. J.; McLaughlin, M. L. *Chem. Commun.* **1998**, 1801–1802. (c) Ousaka, N.; Sato, T.; Kuroda, R. *J. Am. Chem. Soc.* **2008**, *130*, 463–465.

(6) See the Supporting Information.

(7) Manning, M. C.; Woody, R. W. *Biopolymers* **1991**, *31*, 569–586.

(8) A similar pH/pK_a discrepancy has been reported for pH-dependent conformational changes of oligo/polypeptides. See: (a) Zimenkov, Y.; Dublin, S. N.; Ni, R.; Tu, R. S.; Breedveld, V.; Apkarian, R. P.; Conticello, V. P. *J. Am. Chem. Soc.* **2006**, *128*, 6770–6771. (b) Armstrong, K. M.; Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 11337–11340.

(9) The $\Delta\varepsilon_{n-\pi^*}/\Delta\varepsilon_{\pi-\pi^*}$ ratios in MeCN and MeOH were 0.58 (3_{10}-helical)[5c,7] and 1.1 (α-helical),[7] respectively.

(10) Turnbull, D.; Maron, S. H. *J. Am. Chem. Soc.* **1943**, *65*, 212–218.

(11) Rodriguez-Llansola, F.; Escuder, B.; Miravet, J. F. *J. Am. Chem. Soc.* **2009**, *131*, 11478–11484.

JA106118W