ChemComm

RSC Publishing

COMMUNICATION

View Article Online
View Journal | View Issue

Published on 02 September 2013. Downloaded by University of California - Santa Cruz on 23/10/2014 21:21:14.

Cite this: *Chem. Commun.*, 2013, **49**, 10133

Received 23rd June 2013,
Accepted 2nd September 2013

DOI: 10.1039/c3cc44713h

www.rsc.org/chemcomm

Deracemization and the first CD spectrum of a 3_{10}-helical peptide made of achiral α-amino-isobutyric acid residues in a chiral membrane mimetic environment†‡

Francesca Ceccacci,[a] Giovanna Mancini,*[a] Paola Rossi,[b] Paolo Scrimin,*[c] Alessandro Sorrenti[a] and Paolo Tecilla*[b]

Interaction of the racemic helical homo-octapeptide made by the achiral C^{α}-methyl alanine (Aib) amino acid with a chiral enantiopure micellar aggregate made of *N*-dodecylproline led to the deracemization of the helical Aib sequence thus allowing us to obtain for the first time the CD signature in water of a 3_{10} helix devoid of the contribution of any chiral amino acid.

The two most common helical conformations in peptide sequences are the α- and the 3_{10}-helix. The two helices differ in the relative position of C=O and NH involved in hydrogen bond formation ($i \leftarrow i+3$ and $i \leftarrow i+4$ in 3_{10}- and α-helices, respectively).[1] If the α-helix represents the predominant conformation in proteinogenic amino acids,[2] the 3_{10}-helix appears to be the preferred one for sequences rich in α,α-disubstituted amino acids,[3] not only in organic solvents but also in water[4] although the polarity of the solvent appears to control the switch from one conformation to the other, at least in short oligomers.[5] More polar solvents favor the α-helix while less polar ones favor the 3_{10}-helix. α,α-Disubstituted amino acids are strong helicity inducers because the presence of the geminal substituents at the α-carbon limits the conformational freedom of the amino acids as compared to the monosubstituted ones. The smallest member of the family of α,α-disubstituted amino acids is the α-amino-isobutyric acid (Aib or C^{α}-methyl alanine). It has been shown that as little as 5 Aib are sufficient to induce a helical conformation in poly-Aib sequences.[6] Of course the helices formed by these sequences are racemic because Aib, as glycine, is achiral. Furthermore, they interconvert rapidly one into the other[7] thus preventing enantiomer separation. The handedness of a helix is dictated by the configuration of the constituent amino acids: conventional wisdom indicates that L-amino acids induce the formation of a right handed helix while D-amino acids induce that of a left-handed one.[2] It has been shown that the introduction of just one chiral amino acid in an otherwise achiral Aib sequence is sufficient to induce an imbalance in the 1 : 1 equilibrium of the enantiomeric helices.[8] The preferred handedness of the helix depends on the position and structure of the chiral amino acid in the sequence. When this amino acid is placed at the N-terminus, a quaternary amino acid of L-configuration favors a right-handed helix while a regular amino acid of the same configuration favors the opposite handedness;[9] when it is placed at the C-terminus the preferred handedness is less defined.[8a,9–11] It has also been shown that a non-covalent interaction like a charge–charge one between the carboxylate of a Boc-protected chiral amino acid and the protonated N-terminus of a Aib and dehydrophenylalanine sequence breaks the symmetry between the two enantiomeric helices.[12]

However, chirality may also be controlled by the surrounding medium. For instance, in studies concerning the investigation of the control of chirality in biomembrane models it has been observed that aggregates of chiral amphiphilic molecules can deracemize to some extent racemic mixtures of conformational enantiomers such as biphenilic derivatives,[13] bilirubin[14] and helicenes[15] bound to these aggregates. It occurred to us that this could be the case also for the racemic helical mixture of an oligo Aib sequence. If this were true we could be able to prove, for the first time, the possibility of selecting one enantiomer of an amino acid-based helical sequence in a chiral membrane mimetic system and obtaining the electronic circular dichroism (CD) signature of such a helix in the absence of any chiral amino acid covalently bound to it. We report here the very first example of deracemization of a helical 8-mer Aib sequence in a chiral micellar aggregate (Scheme 1).

The H-$(Aib)_8$-O*t*Bu peptide (**1**) was synthesized by conventional solution chemistry following the procedure reported by Toniolo.[16] Peptide **1** was fully characterized by ^{1}H- and ^{13}C-NMR and by ESI-MS and the data are consistent with those reported by Clayden for the same compound obtained by reduction of the corresponding azide[11] (see ESI‡ for synthetic details).

[a] *CNR, Istituto di Metodologie Chimiche, c/o University of Rome "Sapienza", Department of Chemistry, p.le A. Moro 5, I-00165 Roma, Italy. E-mail: giovanna.mancini@uniroma1.it*

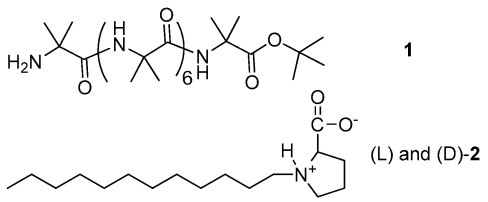
[b] *University of Trieste, Department of Chemical and Pharmaceutical Sciences, via Giorgieri 1, I-34127 Trieste, Italy. E-mail: ptecilla@units.it*

[c] *University of Padova, Department of Chemical Sciences, via Marzolo 1, I-35131 Padova, Italy. E-mail: paolo.scrimin@unipd.it*

† Dedicated to Prof. Claudio Toniolo on the occasion of his retirement.

‡ Electronic supplementary information (ESI) available: Experimental details of synthesis and characterization of peptide **1**, and its IR spectra in $CHCl_3$, and raw CD spectra. See DOI: 10.1039/c3cc44713h

Published on 02 September 2013. Downloaded by University of California - Santa Cruz on 23/10/2014 21:21:14.



Scheme 1 Chemical structures of the octapeptide **1** and of the enantiomeric proline surfactant **2**.

First it was necessary to assess the folded conformation of our peptide sequence, although ample evidence is available in the literature that this would be indeed the case. The helical conformation in peptides is due to the formation of intramolecular hydrogen bonds that, in this particular case, may be as many as six or seven depending on whether the conformation is that of an α- or a 3_{10}-helix, respectively. Formation of these intramolecular H-bonds is evidenced by the appearance, in the IR spectra, of new bands at lower wavelengths both in the NH and C=O stretching regions.[17] The IR spectra of **1** in $CDCl_3$ reveal that these bands are relatively strong and their position is not dependent on concentration (Fig. S1, ESI‡). This confirms that the hydrogen bonds are intramolecular and not intermolecular.

Peptides that are poorly soluble in water are known to bind to micellar aggregates that not only solubilize them but also stabilize their secondary structure.[18] *N*-Dodecylproline, **2**, is known[19] to form spontaneously micellar aggregates above the critical micelle concentration (cmc). Under the conditions of the present experiments the cmc of **2** is 1×10^{-3} M: this means that, in order to create a chiral membrane mimetic environment, we must operate above this concentration. The interaction of **1** with micelles of each enantiomer of **2** was studied by CD. The binding of 0.5 mM **1** with both enantiomers of **2** was investigated in a 25 mM aqueous solution of the surfactant. The choice of the experimental conditions has been a very delicate issue and the conditions used were the result of a fine balance of different aspects such as the optical features of the peptide and the amphiphile, the ratio of their concentration and the cmc of **2**. The 1 : 50 ratio between **1** and **2** ensures that a single peptide is bound to a micelle, preventing peptide aggregation, significant alteration of the aggregate cmc and precipitation. Because the carboxylic group of the surfactant absorbs in the same spectral region of the peptide bond, the CD spectra reported in Fig. 1 were obtained by subtracting the spectrum of the surfactant aqueous solution from that of **1** in the same solution of the surfactant.[20]

Fig. 1 shows that these solutions give rise to relatively strong CD signals in the regions associated with a helical conformation of a peptide sequence.[21] Typically, the standard CD for the 3_{10}-helix is reported to present a minimum at 208 nm and a weaker (40% or less of the main band) shoulder at 222 nm.[22] An α-helix conformation is characterized by a 222 nm band that is equal in intensity or even more pronounced[22,23] than the one at 208 nm. Inspection of Fig. 1 reveals that peptide **1** in the presence of surfactant **2** presents a CD signature consistent with that of a 3_{10} helix. The ratio between the 222 and 208 bands is 0.40–0.45, as shown in Fig. 1. These data confirm the helical conformation of peptide **1** and provide clear evidence that the two enantiomeric

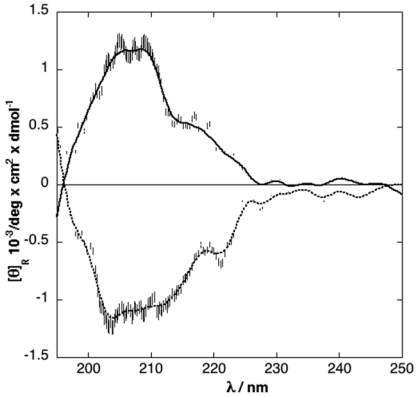


Fig. 1 CD spectrum of 0.5 mM **1** in an aqueous solution of 25 mM **2**; solid line: (D)-**2**, dotted line: (L)-**2**. The data are expressed in terms of $[\theta]_R$, the residue molar ellipticity. The error bars represent the reproducibility in three independent experiments (see ESI‡ for CD data).

helical conformations bind selectively to the two micellized enantiomers of surfactant **2**. Their concentration is, accordingly, amplified by the configuration of the headgroup of the surfactant. Specifically, the surfactant L-enantiomer induces deracemization towards the right handed 3_{10}-helix whereas the D-enantiomer induces, as expected, deracemization towards the left handed 3_{10}-helix (showing an opposite CD spectrum). Since the ellipticity of a single enantiomer of a 3_{10} helical peptide devoid of chiral amino acids is not known we cannot determine the amount of deracemization of **1** resulting from its binding to **2**. Toniolo has shown[4a] that chiral residues increase the ellipticity observed for 3_{10} helical sequences. Accordingly, we can only make cautionary extrapolations from values of known octameric peptides with a single chiral amino acid. Table 1 reports θ_R values at 208 nm for octapeptides with seven Aib and only one L-Leu. The values vary between -3.7×10^3 and -7.5×10^3 deg cm^2 dmol^{-1} depending on the N-protecting group and the solvent suggesting that these parameters may affect both the helical content of the sequences and the preference for the right handed helix. This implies that the extent of deracemization (ee) in our case is at least 33%. This ee is clearly underestimated for several reasons: (i) peptide **1** is not 100% bound to the chiral micellar aggregate; (ii) the peptides in Table 1 are all N-protected while **1** is not: they can hence form an extra H-bond that might contribute to stabilizing the helical conformation; (iii) no chiral amino acid is present in the sequence of **1**. As mentioned above chiral amino acids do contribute to the increase of ellipticity in a sequence.[4a]

It is reasonable to assume that the peptide binds to the micellar aggregate with its polar, protonated N-terminus residing

Table 1 Ellipticity values at 208 nm for octapeptide sequences

Sequence	$-10^{-3}\ \theta_R$ (deg cm^2 dmol^{-1})	Solvent
H(Aib)$_8$O*t*Bu	1.2[a]	Aqueous micelle
Ac(Aib)$_5$-L-Leu-(Aib)$_2$OMe	7.5[b]	MeOH
Ac(Aib)$_5$-L-Leu-(Aib)$_2$OMe	6.2[b]	TFE
Z(Aib)$_5$-L-Leu-(Aib)$_2$OMe	5.4[b]	MeOH
Z(Aib)$_5$-L-Leu-(Aib)$_2$OMe	3.7[b]	TFE

[a] This work. [b] Unpublished data courtesy of Prof. Toniolo and Formaggio.

 This journal is © The Royal Society of Chemistry 2013

Published on 02 September 2013. Downloaded by University of California - Santa Cruz on 23/10/2014 21:21:14.

at the interface with bulk water, interacting with the anionic carboxylate of the proline headgroup of surfactant **2**.[24] This electrostatic interaction would be negligible in water because of the solvation of the ions in this medium but is rather relevant in the confined volume constituted by the interfacial region of the micellar aggregate for two reasons: first the polarity of the environment is lower than that of bulk water, second the local concentration of the ions is much higher.[24] The interaction between the chiral headgroup of the proline-based surfactant would induce the preferred helical sense of the peptide in a similar fashion to what happens when *N*-*t*-butoxycarbamoyl amino acids (Boc-AA-OH) are added to helical Aib/dehydroamino acid-based sequences reported by Inai,[10,12] although in this case chloroform, a much less competitive solvent, is used. The key role played by the free amino group is supported by the failure to observe any deracemization when we use *Z*-protected **1**, although it must be pointed out that the protected peptide is much less soluble under the conditions used. The selection of a right-handed helix with (L)-**2** (and, conversely, a left-handed helix with (D)-**2**) is in full accord with that reported for chloroform by adding Boc-AA-OH to an achiral helical sequence.[10,12,25]

In conclusion, by using a chiral micellar aggregate we were able to deracemize a 3_{10} helical octapeptide made exclusively of the achiral amino acid Aib and to record for the first time its CD spectrum. This constitutes the CD signature of a 3_{10} helix devoid of the contribution of any chiral amino acid. Micellar **2** solubilizes little polar **1** in a local environment of lower polarity than water and contributes the chiral milieu for the deracemization of the two enantiomeric helices, likely through an interaction of the surfactant carboxylate and peptide ammonium groups. In view of the claimed fundamental role of amphiphilic self-assemblies in the origin of life,[26] including the development of homochiral life, we believe that our observation could lend further support to such a hypothesis.

PS is indebted to Prof. C. Toniolo and F. Formaggio for disclosing unpublished material from their own laboratory and for fruitful discussion. Financial support from MIUR (Rome) contract 2010JMAZML is gratefully acknowledged.

Notes and references

1 C. Toniolo, M. Crisma, F. Formaggio, C. Peggion, Q. B. Broxterman and B. Kaptein, *Biopolymers*, 2004, **76**, 162.
2 C.-I. Brandén and J. Tooze, *Introduction to Protein Structure*, Garland Publishing, New York, 1999.
3 (*a*) C. Toniolo, M. Crisma, F. Formaggio, G. Valle, G. Cavicchioni, G. Précigoux, A. Aubry and J. Kamphuis, *Biopolymers*, 1993, **33**, 1061; (*b*) C. Toniolo, M. Crisma, F. Formaggio and C. Peggion, *Biopolymers*, 2001, **60**, 396; (*c*) E. Mossel, F. Formaggio, G. Valle, M. Crisma, C. Toniolo, M. Doi, T. Ishida, Q. B. Broxterman and J. Kamphuis, *Lett. Pept. Sci.*, 1998, **5**, 223.
4 (*a*) F. Formaggio, M. Crisma, P. Rossi, P. Scrimin, B. Kaptein, Q. B. Broxterman, J. Kamphuis and C. Toniolo, *Chem.–Eur. J.*, 2000, **6**, 4498; (*b*) P. Rossi, F. Felluga, P. Tecilla, F. Formaggio, M. Crisma, C. Toniolo and P. Scrimin, *J. Am. Chem. Soc.*, 1999, **121**, 6948; (*c*) P. Rossi, F. Felluga, P. Tecilla, F. Formaggio, M. Crisma, C. Toniolo and P. Scrimin, *Biopolymers*, 2000, **55**, 496; (*d*) C. Sissi, P. Rossi, F. Felluga, F. Formaggio, M. Palumbo, P. Tecilla, C. Toniolo and P. Scrimin, *J. Am. Chem. Soc.*, 2001, **123**, 3169; (*e*) A. Scarso, U. Scheffer, M. Göbel, Q. B. Broxterman, B. Kaptein, F. Formaggio, C. Toniolo and P. Scrimin, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 5144.
5 (*a*) P. Pengo, L. Pasquato, S. Moro, A. Brigo, F. Fogolari, Q. B. Broxterman, B. Kaptein and P. Scrimin, *Angew. Chem., Int. Ed.*, 2003, **42**, 3388; (*b*) M. Bellanda, S. Mammi, S. Geremia, N. Demitri, L. Randaccio, Q. B. Broxterman, B. Kaptein, P. Pengo, L. Pasquato and P. Scrimin, *Chem.–Eur. J.*, 2007, **13**, 407.
6 N. Shamala, R. Nagaraj and P. Balaram, *J. Chem. Soc., Chem. Commun.*, 1978, 996.
7 (*a*) R.-P. Hummel, C. Toniolo and G. Jung, *Angew. Chem., Int. Ed. Engl.*, 1987, **26**, 1150; (*b*) M. Kubasik and A. Blom, *ChemBioChem*, 2005, **6**, 1187; (*c*) M. Kubasik, J. Kotz, C. Szabo, T. Furlong and J. Stace, *Biopolymers*, 2005, **78**, 87.
8 (*a*) B. Pengo, F. Formaggio, M. Crisma, C. Toniolo, G. M. Bonora, Q. B. Broxterman, J. Kamphius, M. Saviano, R. Iacovino, F. Rossi and E. Benedetti, *J. Chem. Soc., Perkin Trans. 2*, 1998, 1651; (*b*) J. Clayden, A. Castellanos, J. Solà and G. A. Morris, *Angew. Chem., Int. Ed.*, 2009, **48**, 5962; (*c*) J. Solà, G. A. Morris and J. Clayden, *J. Am. Chem. Soc.*, 2011, **133**, 3712.
9 R. A. Brown, T. Marcelli, M. De Poli, J. Solà and J. Clayden, *Angew. Chem., Int. Ed.*, 2012, **51**, 1395.
10 Y. Inai, Y. Ishida, K. Tagawa, A. Takasu and T. Hirabayashi, *J. Am. Chem. Soc.*, 2002, **124**, 2466.
11 J. Solà, M. Helliwell and J. Clayden, *J. Am. Chem. Soc.*, 2010, **132**, 4548.
12 Y. Inai, H. Komori and N. Ousaka, *Chem. Rec.*, 2007, **7**, 191.
13 (*a*) S. Borocci, F. Ceccacci, L. Galantini, G. Mancini, D. Monti, A. Scipioni and M. Venanzi, *Chirality*, 2003, **15**, 441; (*b*) F. Ceccacci, G. Mancini, A. Sferrazza and C. Villani, *J. Am. Chem. Soc.*, 2005, **127**, 13762; (*c*) F. Ceccacci, O. Cruciani, M. Diociaiuti, G. Formisano, L. Galantini, W. Lindner, G. Mancini and C. Villani, *Tetrahedron: Asymmetry*, 2006, **17**, 1603; (*d*) A. Alzalamira, F. Ceccacci, D. Monti, S. Levi Mortera, G. Mancini, A. Sorrenti, M. Venanzi and C. Villani, *Tetrahedron: Asymmetry*, 2007, **18**, 1868.
14 (*a*) F. Ceccacci, P. di Profio, L. Giansanti, S. Levi Mortera, G. Mancini, A. Sorrenti and C. Villani, *Tetrahedron: Asymmetry*, 2010, **21**, 2117; (*b*) C. Bombelli, C. Bernardini, G. Elemento, G. Mancini, A. Sorrenti and C. Villani, *J. Am. Chem. Soc.*, 2008, **130**, 2732; (*c*) F. Ceccacci, L. Giansanti, S. Levi Mortera, G. Mancini, A. Sorrenti and C. Villani, *Bioorg. Chem.*, 2008, **36**, 252; (*d*) C. Bernardini, P. D'Arrigo, G. Elemento, G. Mancini, S. Servi and A. Sorrenti, *Chirality*, 2009, **21**, 87.
15 (*a*) K.-I. Yamada, Y. Kobori and H. Nakagawa, *Chem. Commun.*, 2000, 97; (*b*) H. Nakagawa, M. Yoshida, Y. Kobori and K.-I. Yamada, *Chirality*, 2003, **15**, 703; (*c*) H. Nakagawa, M. Onoda, Y. Masuoka and K.-I. Yamada, *Chirality*, 2006, **18**, 212.
16 C. Toniolo, G. M. Bonora, V. Barone, A. Bavoso, E. Benedetti, B. Di Blasio, P. Grimaldi, F. Lelj, V. Pavone and C. Pedone, *Macromolecules*, 1985, **18**, 895.
17 (*a*) C. Toniolo and F. Formaggio, *Chirality*, 2010, **22**, E30; (*b*) H. Maekawa, F. Formaggio, C. Toniolo and N.-H. Ge, *J. Am. Chem. Soc.*, 2008, **130**, 6556.
18 For a recent reference see: T. D. Clark, L. Bartolotti and R. P. Hicks, *Biopolymers*, 2013, **99**, 548.
19 M. Hébrant, P. Burgoss, X. Assfeld and J.-P. Joly, *J. Chem. Soc., Perkin Trans. 2*, 2001, 998.
20 The impossibility to achieve higher concentration of **1** in the micellar environment hampered our attempts to investigate the peptide helix formation using NMR. Other microscopic techniques such as TEM or AFM proved also unsuitable on this regard.
21 C. Toniolo, F. Formaggio and R. W. Woody, Electronic circular dichroism of peptides, in *Comprehensive Chiroptical Spectroscopy: Applications in Stereochemical Analysis of Synthetic Compounds, Natural Products, and Biomolecules*, ed. N. Berova, P. L. Polavarapu, K. Nakanishi and R. W. Woody, John Wiley & Sons, Inc., Hoboken, NJ, USA, vol. 2, 2012.
22 C. Toniolo, A. Polese, F. Formaggio, M. Crisma and J. Kamphuis, *J. Am. Chem. Soc.*, 1996, **118**, 2744.
23 (*a*) G. Yoder, A. Polese, R. A. G. D. Silva, F. Formaggio, M. Crisma, Q. B. Broxterman, J. Kamphuis, C. Toniolo and T. A. Keiderling, *J. Am. Chem. Soc.*, 1997, **119**, 10278; (*b*) R. A. G. D. Silva, S. C. Yasui, J. Kubelka, F. Formaggio, M. Crisma, C. Toniolo and T. A. Keiderling, *Biopolymers*, 2002, **65**, 229.
24 C. A. Bunton, F. Nome, F. H. Quina and L. S. Romsted, *Acc. Chem. Res.*, 1991, **24**, 357.
25 Y. Inai, K. Tagawa, A. Takasu, T. Hirabayashi, T. Oshikawa and M. Yamashita, *J. Am. Chem. Soc.*, 2000, **122**, 11731.
26 (*a*) E. M. Arnett and O. Thompson, *J. Am. Chem. Soc.*, 1981, **103**, 968; (*b*) H. Zepik, E. Shavit, M. Tang, T. R. Jensen, K. Kjaer, G. Bolbach, L. Leiserowitz, I. Weissbuch and M. Lahav, *Science*, 2002, **295**, 1266; (*c*) P. Walde, *Origins Life Evol. Biospheres*, 2006, **36**, 109.