Journal of Molecular Structure 930 (2009) 55–59

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: www.elsevier.com/locate/molstruc

Design of a turn-linker-turn foldamer by incorporating *meta*-amino benzoic acid in the middle of a helix forming hexapeptide sequence: A helix breaking approach

Arpita Dutta [a], Michael G.B. Drew [b], Animesh Pramanik [a,*]

[a] Department of Chemistry, University of Calcutta, 92, A. P. C. Road, Kolkata, West Bengal 700 009, India
[b] School of Chemistry, The University of Reading, Whiteknights, Reading RG6 6AD, UK

ARTICLE INFO

Article history:
Received 18 March 2009
Received in revised form 22 April 2009
Accepted 24 April 2009
Available online 5 May 2009

Keywords:
Peptide
β-Turn
Foldamer
Self-assembly
Duplex
Channels

ABSTRACT

Single crystal X-ray diffraction studies reveal that the incorporation of *meta*-amino benzoic acid in the middle of a helix forming hexapeptide sequence such as in peptide **I** Boc-Ile(1)-Aib(2)-Val(3)-*m*-ABA(4)-Ile(5)-Aib(6)-Leu(7)-OMe (Aib: α-amino isobutyric acid; *m*-ABA: *meta*-amino benzoic acid) breaks the helix propagation to produce a turn-linker-turn (T-L-T) foldamer in the solid state. In the crystalline state two conformational isomers of peptide **I** self-assemble in antiparallel fashion through intermolecular hydrogen bonds and aromatic π–π interactions to form a molecular duplex. The duplexes are further interconnected through intermolecular hydrogen bonds to form a layer of peptides. The layers are stacked one on top of the other through van der Waals interactions to form hydrophilic channels filled with solvent methanol.

1. Introduction

β-Turns which were first recognized by Venkatachalam in the late 1960s [1], are found to play an important role in stabilizing tertiary structures, initiating folding and facilitating intermolecular recognition [2–4]. Because of their critical importance there has been considerable interest in designing β-turns and β-turn mimetics [5–14]. Although there are several examples of the design and stabilization of isolated β-turns, there are few examples in the literature of small acyclic peptides containing more than one β-turn [15,16]. The peptide turn-linker-turn (T-L-T) foldamer will be useful in designing biologically active peptides. Previously it has been shown that a T-L-T foldamer can be designed and stabilized by connecting two turn inducing tripeptides with a flexible linker such as 1,2-ethylenediamine [15]. The T-L-T foldamers so formed were found to fabricate three-dimensional framework of channel in the solid state through self-assembly.

In this report we explore the possibility of generating T-L-T foldamer by a helix breaking approach. It is known that the 3_{10} helical structures of peptides containing Aib (α-amino isobutyric acid) are stabilized by successive 4 → 1 CO···NH hydrogen bonds, with idealized φ, ψ values of −60°, −30° characteristic of the right handed screw [17–20]. Essentially several turns are connected along the helix axis in a continuous fashion to form the helical structure. Therefore the breaking of helix propagation by inserting a suitable linker in the middle of a helix forming sequence may produce a T-L-T foldamer. Earlier attempts incorporating flexible linkers such as the dipeptide fragments (-β-Ala-γ-amino butyric acid-) and ω-amino acid such as δ-amino valeric acid in the middle of helix forming sequences failed to break the helix propagation [21,22]. The results showed that in spite of losing few hydrogen bonding donors and acceptors in the middle of the sequence the linkers are nicely accommodated into the helical structures. Therefore we thought that instead of using a flexible linker, the incorporation of a rigid linker such as *meta*-amino benzoic acid (*m*-ABA), a substituted γ-amino butyric acid with an all *trans* extended configuration in the middle of a helix forming sequence would help to break the helix propagation. Keeping this in view we chose the peptide **I** Boc-Ile(1)-Aib(2)-Val(3)-*m*-ABA(4)-Ile(5)-Aib(6)-Leu(7)-OMe (Fig. 1) to examine the formation of the T-L-T motif from expected helix breaking. It is important to note that hexa-peptides containing Aib at position 2 and 5 adopt a mixed $3_{10}/\alpha$-helical structure in the solid state and well developed homogeneous 3_{10} helical conformation in the solution phase [23]. Peptide **I** was synthesized by conventional solution phase methods. The solid state structure of the peptide was determined by single-crystal X-ray diffraction studies.

2. Experimental

2.1. Synthesis of the peptide *I*

The peptide was synthesised by conventional solution phase procedures using a racemization free fragment condensation strat-

* Corresponding author. Tel.: +91 33 2484 1647; fax: +91 33 2351 9755.
E-mail address: animesh_in2001@yahoo.co.in (A. Pramanik).

0022-2860/$ - see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.molstruc.2009.04.037

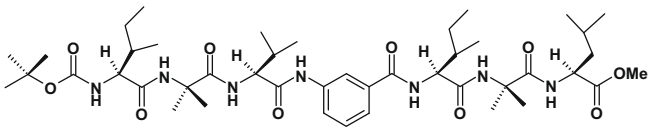

Fig. 1. Schematic diagram of peptide **I**.

egy [24]. The *t*-butyloxycarbonyl and methyl ester group were used for amino and carboxyl protections, respectively, and *N*,*N*′-dicyclohexylcarbodiimide (DCC)/1-hydroxybenzotriazole (HOBT) as coupling agents. Deprotections were performed using trifluoroacetic acid or saponification, respectively. Methyl ester hydrochlorides of Aib, Leu and Val were prepared by the thionyl chloride–methanol procedure. All the intermediates obtained were checked for purity by thin layer chromatography (TLC) on silica gel and used without further purification. The final peptide was purified by column chromatography using silica gel (100–200 mesh) as the stationary phase and ethyl acetate and petroleum ether mixture as the eluent. The reported peptide was fully characterized by NMR studies and X-ray crystallography.

*2.1.1. Boc-Ile-Aib-Val-OMe (**1**)*

The peptide was prepared using the literature method [25].

*2.1.2. Boc-Ile-Aib-Val-OH (**2**)*

Compound **1** (1.4 g, 3.16 mmol) was dissolved in methanol (15 ml) and 2 M NaOH (5 ml) was added. The reaction mixture was stirred at room temperature for 2 days. The progress of the reaction was monitored by TLC. After completion of the reaction the methanol was evaporated. The residue obtained was diluted with water and washed with diethylether. The aqueous layer was cooled in an ice-bath and neutralized with 2 M HCl and then extracted with ethyl acetate. The solvent was evaporated *in vacuo* to give a white solid. Yield: 1.3 g (95.8%).

*2.1.3. Boc-Ile-Aib-Val-m-ABA-OMe (**3**)*

Compound **2** (1.2 g, 2.79 mmol) was dissolved in DMF (5 ml). *m*-ABA-OMe obtained from its hydrochloride (1.05 g, 5.6 mmol) was added, followed by DCC (0.86 g, 4.2 mmol) and HOBT (0.38 g, 2.79 mmol). The reaction mixture was stirred at room temp for 3 days. The precipitated *N*,*N*′-dicyclohexylurea (DCU) was filtered and to the filtrate 20 ml of ethyl acetate was added. The organic layer was washed with 1 N HCl (3 × 30 mL), 1 M Na_2CO_3 solution (3 × 30 mL) and water. The solvent was then dried over anhydrous Na_2SO_4 and evaporated *in vacuo,* giving a light yellow gum. Yield: 1.4 g (91.6%). Purification was carried out using silica gel as the stationary phase and ethyl acetate–petroleum ether mixture as the eluent. Mp = 178–180 °C; IR (KBr): 3420, 3302, 1724, 1665, 1604, 1547, 1511 cm^{-1}; ^1H NMR 300 MHz ($CDCl_3$, δ ppm): 9.12 (*m*-ABA NH, 1H, *s*); 8.49 (*m*-ABA (4) H_a, 1H, *s*); 8.17 (*m*-ABA (4) H_d, 1H, *d*, *J* = 6.9 Hz); 7.75(*m*-ABA (4) H_b, 1H, *d*, *J* = 7.8 Hz); 7.36 (*m*-ABA (4) H_c, 1H, *t*, *J* = 8.1 Hz); 6.95 (Val (1) NH, 1H, *d*, *J* = 8.4 Hz); 6.68 Aib(2) NH, 1H, *s*); 5.04 (Ile (3) NH, 1H, *d*, *J* = 3.9 Hz); 4.58–4.54 ($C^{\alpha}H$ of Val (3), 1H, *m*); 3.91 (–OCH_3, 3H, *s*); 3.89–3.84 ($C^{\alpha}H$ of Ile(1), 1H, *m*); 2.70–2.65 ($C^{\beta}Hs$ of Val (3), 1H, *m*); 1.95-1-93 ($C^{\beta}Hs$ of Ile (1), 1H, *m*);1.55 ($C^{\beta}Hs$ of Aib, 6H, *s*);1.44 (Boc–CH_3s, 9H, *s*); 1.29–1.27 ($C^{\gamma}Hs$ of Ile (1) , 5H, *m*); 1.01–0.92 ($C^{\delta}Hs$ of Ile (1) and $C^{\gamma}Hs$ of Val (3), 9H, *m*); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 173.9, 171.9, 170.1, 167.1, 156.5, 138.9, 130.5, 128.6, 124.9, 124.5, 121.1, 81.2, 60.7, 59.3, 57.2, 51.9, 36.4, 29.1, 28.1, 27.5, 25.3, 24.0, 19.5, 16.9, 15.74, 11.52; HR–MS (M^+Na^+) = 571.31, M_{calcd} = 548.67.

*2.1.4. Boc-Ile-Aib-Val-mABA-OH (**4**)*

Compound **3** (1.2 g, 2.19 mmol) was dissolved in methanol (15 ml) and 2 M NaOH (5 ml) was added. The reaction mixture was stirred at room temperature for 2 days. The progress of the reaction was monitored by TLC. After completion of the reaction the methanol was evaporated. The residue obtained was diluted with water and washed with diethylether. The aqueous layer was cooled in an ice-bath and neutralized with 2 M HCl and then extracted with ethyl acetate. The solvent was evaporated *in vacuo* to give a white solid. Yield: 1.1 g (94.1%).

*2.1.5. Boc-Ile-Aib-Leu-OMe (**5**)*

This peptide was prepared following the literature method [25].

Boc-Ile-Aib-Val-m-ABA-*Ile-Aib-Leu-OMe* (Peptide **I**): To *Boc-Ile-Aib-Leu-OMe* (***5***), (0.47 g, 1.07 mmol) trifluoroacetic acid (3 ml) was added at 0 °C and stirred at room temperature. The removal of the Boc-group was monitored by TLC. After 3 h the trifluoroacetic acid was removed under reduced pressure to afford the crude trifluoroacetate salt. The residue was taken up in water and washed with diethyl ether. The pH of the aqueous solution was adjusted to eight with sodium bicarbonate and extracted with ethyl acetate. The extracts were pooled, washed with saturated brine, dried over sodium sulfate, and concentrated to a highly viscous liquid that gave a positive ninhydrin test. This free base of the tripeptide was added to a well ice-cooled solution of compound **4** (0.57 g, 1.07 mmol) in DMF (6 ml) followed by DCC (0.33 g, 1.60 mmol) and HOBt (0.17 g, 1.28 mmol). The reaction mixture was stirred at room temperature for 4 days. The residue was taken up in ethyl acetate and DCU was filtered off and to the filtrate 20 ml of ethyl acetate was added. The organic layer was washed with 2 M HCl (3 × 50 ml), 1 M Na_2CO_3 solution (3 × 50 ml) and brine, dried over anhydrous Na_2SO_4 and evaporated *in vacuo*, to yield a white solid. Purification was done using silica gel as stationary phase and ethyl acetate–petroleum ether mixture as the eluent. Single crystals were grown from methanol and ethyl acetate mixture by slow evaporation and were stable at room temperature.

Yield: 0.81 g (88.3%). Mp = 150–152 °C; IR (KBr): 3313, 1717, 1674, 1526 cm^{-1}; ^1H NMR 300 MHz (DMSO-d_6, δ ppm): 9.96 (*m*-ABA(4) NH, 1H, *s*); 8.37 (*m*-ABA(4) H_d, 1H, *d*, *J* = 6.6 Hz); 8.34 (*m*-ABA(4) H_a, 1H, *s*); 8.08 (*m*-ABA(4) H_b and Aib (6) NH, 2H, *bs*); 7.74 (Leu(7) NH, 1H, *d*, *J* = 7.8 Hz); 7.57 (Ile(5) NH, 1H, *d*, *J* = 7.8 Hz); 7.33 (*m*-ABA(4) H_c, Aib (2) NH, 2H, *m*); 7.07 (Val(3) NH, 1H, *d*, *J* = 8.4 Hz); 6.76 (Ile(1) NH, 1H, *d*, *J* = 7.5 Hz); 4.26–4.23 ($C^{\alpha}Hs$ of Ile(5) and Leu (7), 2H, *m*); 4.12–4.07 ($C^{\alpha}H$ of Val (3),1H, *m*); 3.80–3.76 ($C^{\alpha}H$ of Ile(1),1H, *m*); 3.57 (–OCH_3, 3H, *s*); 1.59–2.15 ($C^{\beta}Hs$ of Ile(1), Val(3), Ile(4) and Leu(6), 5H, *m*); 1.56 ($C^{\beta}Hs$ of Aib(6), 6H, *s*); 1.53 (Boc-CH_3s, 9H, *s*); 1.45 ($C^{\beta}Hs$ of Aib(2), 6H, *s*); 1.30–1.25 ($C^{\gamma}Hs$ of Ile(1), Ile(5) and Leu(6), 11H, *m*); 1.0–0.87 ($C^{\gamma}Hs$ of Val(3), $C^{\delta}Hs$ of Ile(1), Ile(5) and Leu(7), 18H, *m*); ^{13}C NMR 75 MHz (DMSO-d_6, δ ppm): 173.93, 173.83, 173.02, 171.70, 171.27, 170.08, 167.03, 155.71, 138.80, 134.62, 128.47, 122.47, 122.19, 119.01, 78.19, 56.28, 56.09, 51.79, 50.24, 36.23, 34.97, 30.62, 28.19, 26.94, 25.73, 25.31, 24.69, 24.25, 23.72, 23.08, 22.41, 21.19, 19.21, 17.97, 15.40, 15.12, 11.10, 10.57; HR–MS (M^+Na^+) = 882.03, M_{calcd} = 860.11.

2.2. FTIR spectroscopy

IR spectrum of peptide **I** was examined using Perkin Elmer-782 model spectrophotometer. The solid state FTIR measurements were performed using the KBr disk technique.

2.3. NMR experiments

All ^1H and ^{13}C NMR spectra of peptide **I** were recorded on Bruker Avance 300 model spectrometer operating at 300 and 75 MHz, respectively. The peptide concentrations were 10 mM in $CDCl_3$ for ^1H NMR and 40 mM in $CDCl_3$ for ^{13}C NMR.