183. An Example of Head-to-Head Dimerization of $\beta^{4.4}$-Helices

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Summary

^{1}H-NMR and vapor-pressure osmometry results are presented, which indicate the occurrence of a rapid equilibrium involving the head-to-head dimerization of $\beta^{4.4}$-helices in chloroform solutions of HCO-L-Ile-(D-aIle-L-Ile)$_4$-OMe. This equilibrium typifies the one that, in *Urry*'s view, would be responsible for the formation and breaking down of the ion-conducting channels formed by gramicidin A in lipid bilayers.

Introduction. – Several studies [1] [2] have indicated that the ion-conducting transmembrane channels formed by gramicidin A in lipid bilayers originate through a dimerization process, and *Urry* [3] [4] has proposed that the channels are helical dimers consisting of two single-stranded β-helices connected head-to-head (formyl-end-to-formyl-end). These helices should be [4] $\beta^{6.3}$-helices (the superscript indicates the approximate number of residues per turn), but also $\beta^{4.4}$-helices [3] and $\beta^{8.2}$-helices [4] [5] have been considered. *Urry*'s proposal is consistent with a number of experimental observations [6], but so far the ability of single-stranded β-helices to give head-to-head dimers has not been demonstrated either with gramicidin A or with other *N*-formyl peptides. We present here results of a ^{1}H-NMR study of the stereo-co-oligopeptide [7] HCO-L-Ile-(D-aIle-L-Ile)$_4$-OMe (**2**) in $CDCl_3$ at 25° which provide the first example of a head-to-head dimerization of $\beta^{4.4}$-helices in solution. Our interpretation has been aided by a similar study of Boc-L-Ile-(D-aIle-L-Ile)$_4$-OMe (**1**), an analog that cannot give head-to-head β-helical dimers owing to the bulkiness of the Boc-group. The observations made with **1** are presented first.

Results and Discussion. – At 25° and at least up to a concentration of 97.2 mg/ml (highest concentration used) **1** occurs in chloroform as non-associated right-handed $\beta^{4.4}$-helices. This conclusion is based on the following observations. The NMR-spectral features do not depend on concentration, and the measured molar mass is that of a monomer. The nine NH signals *(Fig. 1, (a))* are distributed over a broad spectral region (from 5.3 ppm to 8.7 ppm), as one would expect for the case of only one or a few interconverting structures with common H-bonding characteristics. The values of the coupling constants ^3J(NH,C$^\alpha$H) are in the range 8–10 Hz, pointing to β-helices [3] [8]. When considered in the light of the correlations found [9–11] with β-helical oligovalines in $CDCl_3$, the location of the NH-signals is such to rule out all monomeric

β-helices except right-handed $\beta^{4.4}$-helices. These correlations point to β-helices having a non-H-bonded urethane-NH (NH(1); signal at 5.32 ppm in *Fig. 1, (a)*), two non-H-bonded amide-NH's (signals at 6.5–6.8 ppm) and six H-bonded amide-NH's (signals at more than 7.5 ppm from TMS). Right-handed $\beta^{4.4}$-helices of **1** are the only ones to present this specific H-bonding situation. The two amide-NH's not engaged in H-bonding are those of the third and eighth residue (NH(3) and NH(8)) of the peptide chain.

Differing from **1** only in the nature of a terminal group, **2** should also be β-helical in chloroform. That this is indeed the case is shown for instance by the fact that the coupling constants ^3J(NH,C$^\alpha$H) observed for **2** are also in the range 8–10 Hz. However, the behavior of the formyl nonapeptide is somewhat different from that of the Boc-analog. Thus vapor-pressure osmotic measurements have yielded apparent molar masses between 1 and 2 times the formula mass of **2** (1078.49), indicating that the

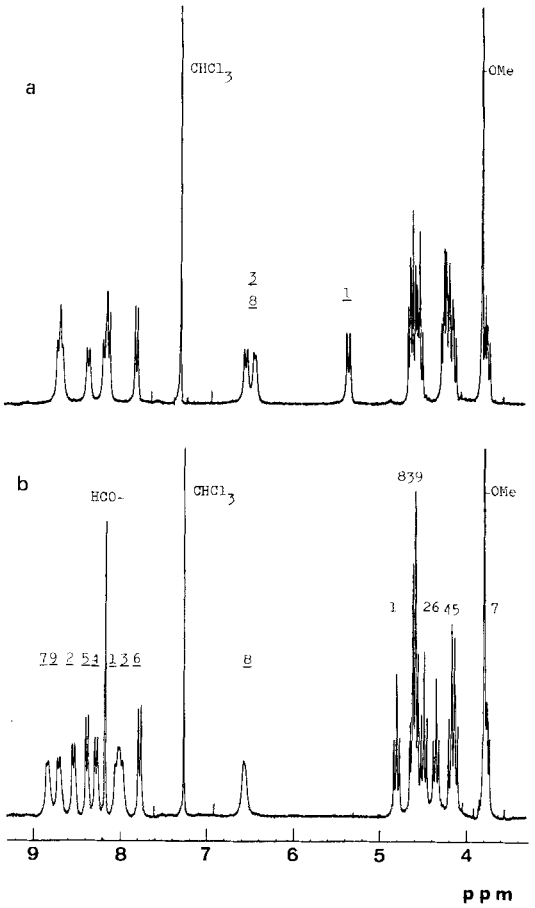

Fig. 1. *NH- and C$^\alpha$H-regions of a 300-MHz ^{1}H-NMR spectrum of* **1** (a) *and of* **2** (b) *in $CDCl_3$ at 25°*. Concn., 20.6 mg/ml (**1**) and 39.3 mg/ml (**2**). The numbers identify the residues responsible for the backbone proton resonances that have been assigned. Those related to NH resonances are underlined.

monomer is accompanied by an aggregate – most likely a dimer. Consistent with this, the positions of several NMR signals given by **2** in $CDCl_3$ change when varying the solution concentrations. Among the NH-resonances, the most mobile signals by far are the two which overlap near 8.0 ppm in the spectrum shown for **2** in *Fig. 1*. This pair of signals has been observed at 7.2–7.4 ppm at the lowest (4.2 mg/ml) concentration used and at 8.3–8.5 ppm at the highest (61.2 mg/ml). Therefore, the groups associated with these signals should be H-bonded in the aggregate but not in the monomer. The signal near 6.6 ppm and the singlet at 3.79 ppm *(Fig. 1, (b))* are among those which do not appear to be affected by the concentration; consequently the environment of the associated groups, a non-H-bonded amide-NH and the OMe, should be nearly the same in the monomer and in the aggregate. It is also important to note that the position of the OMe singlet is the same for **2** as for **1**. We have assigned all backbone proton resonances of **2** by combining results of decoupling experiments, used to correlate signals of the same residue and nuclear *Overhauser* effect (NOE) measurements used to correlate the signals of the $C^{\alpha}H$-proton of a residue and of the NH-proton of the residue immediately following. This is possible for β-helices since these protons are close to each other [12]. Irradiation of the NH(1) signal causes the collapse of the narrow

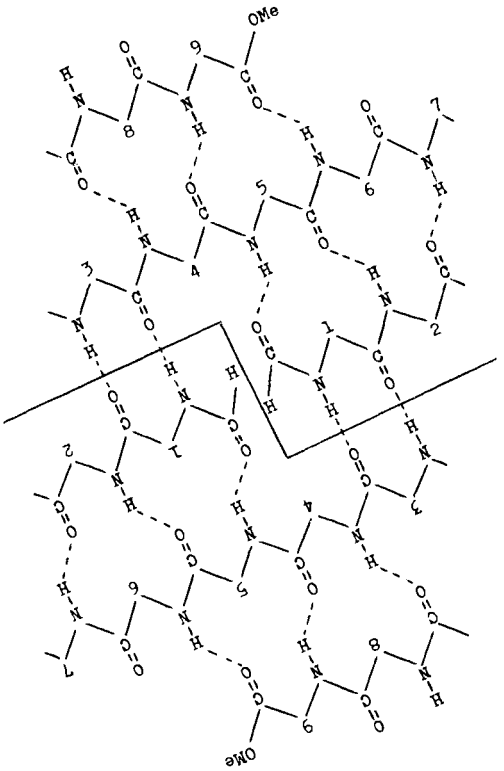

Fig. 2. *Scheme of the right-handed, head-to-head β-helical dimer of* **2**. Represented is a dimer that has been oriented with the helix axis parallel to the long border of the page, split along the back in this direction, opened and flattened. The line in the middle serves to distinguish between the two monomeric helices. The $C^{\alpha}H$ groups and the lateral substituents are not shown; the numbers give the position of the residues in the chain.

(^3J(HCO,NH(1)) < 2 Hz) HCO-doublet to a singlet, and we have used this criterium to check the identity of the NH(1) signal. The results of the assignment show *(Fig. 1, (b))* that the two overlapping signals near 8.0 ppm are those of NH(1) and NH(3), and that the signal near 6.6 ppm is that of NH(8). Based on this evidence, we conclude that **2** occurs in chloroform as right-handed $\beta^{4.4}$-helices and as dimers formed by the head-to-head association of two such helices *(Fig. 2)*, and that there is rapid interconversion between the two species. Note that the NH(1)- and NH(3)-groups that are free in the non-associated helices are H-bonded in the dimer, being used for the connection, and that the NH(8) remains free upon dimerization. The dimer has a C_2-symmetry axis perpendicular to the axis of the helices and passing through the two formyl H's.

Russian authors [13] have calculated the amide-I-band frequencies of different types of β-helical structures for gramicidin A and analogs of shorter chain length. For the $\beta^{4.4}$-helical structure of a nonapeptide they have obtained 1659 cm^{-1} (monomeric helices) and 1650 cm^{-1} (head-to-head dimer). Our IR measurements on $CHCl_3$ solutions of **1** and **2** have given as band position for the two situations 1654 cm^{-1} and 1644 cm^{-1}.

Concluding Remarks. – By providing a pertinent example, this study justifies the notion that in a suitable medium, single-stranded β-helices of formyl peptides may associate linearly to form head-to-head dimers. The example concerns helices ($\beta^{4.4}$-helices) considered [4] to be too narrow for functioning as ion-conducting channels. A system involving $\beta^{6.3}$-helices would model closely the one advocated by *Urry* [6] for gramicidin A in membranes, and we are considering ways to realize and to study it.

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Experimental. – *Syntheses.* The peptide **1** was prepared by a stepwise procedure using conditions similar to those used [14] for synthesizing Boc- and OMe-protected oligo-L-isoleucines and oligo-D-alloisoleucines, and was purified by recrystallization from MeOH/H_2O. Anal. calc. for $C_{60}H_{111}N_9O_{12}$: C 62.63, H 9.72, N 10.96, C/N 5.71; found: C 61.56, H 9.56, N 10.62, C/N 5.79.

The peptide **2** was obtained as follows. Compound **1** (88 mg, 0.076 mmol) was treated with HCOOH (1.8 ml, 48 mmol) at 50° for 1 h. The solution was then cooled to r.t. and 0.65 ml (6.9 mmol) of Ac_2O were added. After stirring for 4 h, the mixture was evaporated; the residue was dissolved in $CHCl_3$ and this solution was washed with 0.5N Na_2CO_3, with H_2O, and dried ($MgSO_4$). Evaporation of the solvent and recrystallization of the residue from $CHCl_3$/MeOH yielded analytically pure **2**. Anal. calc. for $C_{56}H_{103}N_9O_{11}$: C 62.37, H 9.63, N 11.69; found: C 62.95, H. 9.44, N 10.95.

Measurements. The vapor-pressure osmometry measurements were carried out at 25° with a *Wescan* model *232* instrument. Calibration was done with benzil. The ^{1}H-NMR measurements were carried out with a *Bruker AM-300* spectrometer. The sample temperature was 25°. Chemical shifts are relative to internal TMS.

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