83. Sequence Dependence of Secondary-Structure Formation

Part IV[1])

Helix-Forming Potential of Amphiphilic Oligopeptides Containing Aib Residues

by **Manfred Mutter***, **Karl-Heinz Altmann**, **Andreas Flörsheimer**, and **Jürgen Herbert**

Institut für Organische Chemie der Universität Basel, St. Johanns-Ring 19, CH–4056 Basel

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The conformational properties of Aib-containing oligopeptides having the propensity to adopt amphiphilic helical conformations were investigated by CD spectroscopy in solution. The peptides CF_3COOH · H-Pro-Glu-[Ala-Aib-Glu-Aib-]$_4$Gly-OH (**I**), HCl · H-Pro-Ala-Aib-[Glu-Ala-Ala-Aib-]$_2$Glu-Ala-Aib-Gly-PEGM (**II**), and CF_3COOH · H-Ala-Aib-[Glu-Glu-Ala-Aib-]$_3$PEGM (**III**) were synthesized according to the general principles of the liquid-phase method for peptide synthesis. Peptides **I**–**III** exhibit helical conformations in CF_3CH_2OH, MeOH, and in H_2O at acidic pH; however, at pH 7, only **II** forms a stable helix, whereas **I** and **III** are predominantly in an unordered conformation. Some general features for the construction of amphiphilic helices are discussed in the light of the experimental data.

Introduction. – The conformational properties of Aib-containing peptides[2]) have been object of extensive studies over the last few years. These investigations comprise biologically active molecules as alamethicin and suzukacillin as well as model peptides with varying Aib content [2–7].

In agreement with theoretical considerations [8] [9], these studies revealed a strong secondary-structure-inducing effect of the sterically constrained Aib residue. For example, the incorporation of Aib in short peptide sequences of up to 6 amino-acid residues results in the formation of β-turns [7], whereas a strong helix-inducing effect is observed for longer peptide chains [5] [6]. Recent studies have shown that the insertion of one single Aib residue in a β-structure-forming oligopeptide induces a conformational transition of the type β-structure→α-helix [10].

The unique properties of Aib qualify this residue as a powerful tool for the design of peptide sequences exhibiting tailor-made conformational and physico-chemical features. Most notably, the construction of stable amphiphilic helices should be possible by incorporation of a sufficient number of Aib residues.

Helices with amphiphilic character have attracted a lot of attention in the modelling of the overall amphiphilicity of peptide hormones [11] [12]. In our ongoing studies concerning the sequence dependence of secondary-structure formation, we have recently focussed our interest upon the construction of polypeptides which fold in a specific

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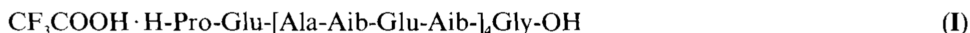
[1]) Part III: [1].

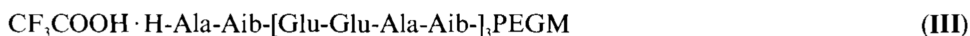
[2]) Abbreviations used according to IUPAC/IUB; Aib, α-aminoisobutyric acid; DCC, dicyclohexylcarbodiimide; DMF, *N,N*-dimethylformamide; HOBt, 1-hydroxy-1*H*-benzotriazole; Fmoc, 9-fluorenylmethoxycarbonyl; PEGM, polyethyleneglycol monomethyl ether (M_r = $5 \cdot 10^3$); Su, succinimidyl.

tertiary structure ('artificial proteins'). As shown previously [13], the availability of amphiphilic peptide blocks with the propensity to adopt stable secondary structures are a basic element of the construction of new proteins. Thus, the investigation of amphiphilic model peptides with a high helix-forming potential is of fundamental importance for the achievement of the indicated goals.

In the present paper, the following peptide sequences are studied by CD spectroscopy in various solvents:


CF_3COOH · H-Pro-Glu-[Ala-Aib-Glu-Aib-]$_4$Gly-OH **(I)**

HCl · H-Pro-Ala-Aib-[Glu-Ala-Ala-Aib-]$_2$Glu-Ala-Aib-Gly-PEGM **(II)**


CF_3COOH · H-Ala-Aib-[Glu-Glu-Ala-Aib-]$_3$PEGM **(III)**

By adoption of an α-helical conformation, the amphiphilic properties of these peptides are demonstrated for peptide **II** by means of the helical wheel [14]. As can be seen from *Fig. 1*, the polar (Glu) and apolar (Aib, Ala) residues are located on different sides of the wheel, resulting in an amphiphilic structure of the helical cylinder.

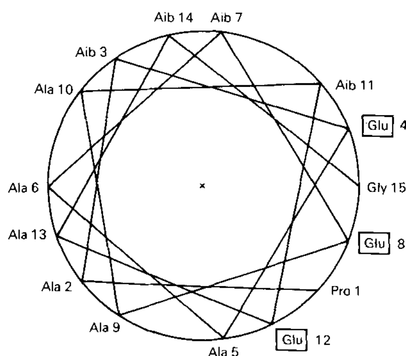


Fig. 1. *Schematic representation of the amphiphilicity of peptide* **II** *when adopting an α-helical conformation by means of the helical wheel* [14]

Peptide synthesis followed the general principles of the liquid-phase method with polyethylene glycol as solubilizing polymeric support [15]. To account for any difficulties arising from couplings to the sterically hindered Aib [16], this amino acid was incorporated as the C-terminal residue of a protected dipeptide in each case.

Previous studies have shown that PEGM exerts no significant influence upon the preferred conformation of the attached peptide in solution [17]. For this reason, the conformational properties of peptides **II** and **III** were investigated making use of the solubilizing effect of PEG.

Results and Discussion. – The CD spectra of peptides **I–III** in CF_3CH_2OH are shown in *Fig. 2*.

They are all characterized by two negative *Cotton* effects around 220 nm (n-π^* transition) and 205 nm (π-π^* transition), which is typical for peptides in a helical conformation. With respect to the intensity of the two bands, the ratio $R = [\theta]_{n\text{-}\pi^*}/[\theta]_{\pi\text{-}\pi^*}$ decreases in the series **I** > **II** > **III** *(Table)*; the value for peptide **III** ($R = 0.61$) is signi-

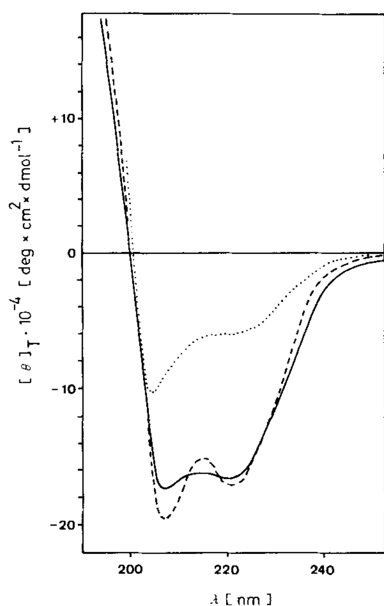


Fig. 2. *CD spectra of peptides* **I** (——), **II** (– – –), *and* **III** (· · · ·) *in* CF_3CH_2OH

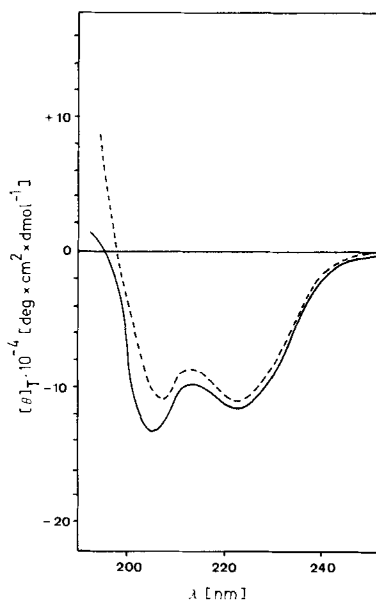


Fig. 3. *CD spectra of peptide* **II** *in* H_2O *at pH 7* (——) *and pH 1* (– – –)

Table. *CD-Spectroscopic Properties of Peptides* **I–III** *in Different Solvents*

Peptide	Solvent	$\lambda_{n-\pi^*}$[a])	$[\theta]_{n-\pi^*} \times 10^{-5}$[b])	$\lambda_{\pi-\pi^*}$[c])	$[\theta]_{\pi-\pi^*} \times 10^{-5}$[d])	R[e])
I	CF_3CH_2OH	220	−1.64	206	−1.75	0.94
II	CF_3CH_2OH	221	−1.60	207	−1.99	0.80
III	CF_3CH_2OH	220	−0.62	204	−1.02	0.61
I	MeOH	223	−1.95	208	−1.92	1.02
II	MeOH	223	−1.73	208	−2.19	0.79
III	MeOH	220	−0.56	204	−0.94	0.60
I	H_2O, pH 7	226	−0.49	205	−1.42	0.34
II	H_2O, pH 7	223	−1.15	205	−1.32	0.87
III	H_2O, pH 7	225	−0.29	201	−1.01	0.29
I	H_2O, pH 1	223	−1.38	208	−1.25	1.10
II	H_2O, pH 1	223	−1.13	207	−1.10	1.03
III	H_2O, pH 2	225	−0.49	203	−1.10	0.45

[a]) Wavelength of the n-π* transition in nm.
[b]) Total molar ellipticity $[\theta]_T$ at $\lambda_{n-\pi^*}$ expressed in deg · cm² · dmol⁻¹.
[c]) Wavelength of the π-π* transition in nm.
[d]) Total molar ellipticity $[\theta]_T$ at $\lambda_{\pi-\pi^*}$ expressed in deg · cm² · dmol⁻¹.
[e]) $R = [\theta]_{n-\pi^*}/[\theta]_{\pi-\pi^*}$.

ficantly lower than for **I** ($R = 0.94$) and **III** ($R = 0.80$). It has been proposed earlier that R is *ca.* 1 for α-helical polypeptides [18]; however, recent studies indicate that much lower values are possible for α-helix-forming oligopeptides containing Aib [5] [2]. For example, R is 0.695 for the 11-residue peptide Boc-Ala-[Ala-Aib-]$_2$Glu(OBzl)-Ala-[Ala-Aib-]$_2$OMe in CF_3CH_2OH and 0.77 for alamethicin in the same solvent. In both cases, an α-helical

conformation has been established in the solid state by X-ray diffraction studies. Furthermore, Aib-containing oligopeptides forming 3_{10}-helices may show *R* values of *ca.* 0.5 only, as demonstrated for Boc-[Aib-Ala-]$_5$OMe in CF_3CH_2OH and in MeOH [19].

In view of these findings, it can be concluded that all three peptides exist predominantly in a helical conformation in CF_3CH_2OH. Although no unequivocal information is available from CD spectra with regard to the type of helix at hand, the high *R* values suggest the onset of an α-helical conformation for peptides **I** and **II**. On the other hand, the lower ratio $[\theta]_{n-\pi^*}/[\theta]_{\pi-\pi^*}$ for peptide **III** is an indication for a predominantly 3_{10}-helical structure. The high Aib content of this peptide in addition to the strong helix-promoting properties of CF_3CH_2OH make it very unlikely that high amounts of unordered conformations are present under the experimental conditions. Consequently, the low *R* value of **III** is more consistent with the presence of a highly developed 3_{10}-helix. The origin of this different behaviour of **III**, especially when compared to **II** having approximately the same chain-length and Aib content, is very difficult to rationalize at present. Possibly, H-bond interactions between adjacent Glu residues make a 3_{10}-helical conformation more favourable for peptide **III**.

Going from CF_3CH_2OH to *MeOH* as solvent, the minima in the CD spectra of peptides **I** and **II** are slightly red-shifted, accompanied by a small increase in ellipticities for both peptides *(Table)*. For peptide **III**, no minima shift is observed; similar to the findings in CF_3CH_2OH, ellipticities are significantly lower for this peptide. From the CD spectra only minor differences between the conformation in CF_3CH_2OH and MeOH can be deduced for all three peptides. It should be mentioned that the higher *R* value for **I** in MeOH *(Table)* points to a possible aggregation in this solvent. Interestingly, this effect is only observed for the free peptide **I**, whereas for the PEG-bound peptides **II** and **III**, *R* remains nearly constant *(Table)*.

The most unusual results were obtained in aqueous solution. In *Figs. 3–5,* the CD spectra of **I–III** in *water* at neutral pH are shown. It is obvious that only peptide **II** *(Fig. 3)* is able to adopt a well developed helical conformation under these conditions (negative *Cotton* effects at 205 and 223 nm, *Table*). On the other hand, peptides **I** and **III** are predominantly unordered at pH 7 as indicated by low *R* values *(Table)*; however, a small amount of ordered conformation in the conformational equilibrium cannot be excluded. The disruption of the helical structure can be rationalized by electrostatic repulsions between charged Glu side-chains for peptide **III**. This result is in agreement with the conformational behaviour of monodispersed homopolymers of glutamic acid ((L-Glu)$_n$), existing exclusively in an unordered conformation up to $n = 20$ at neutral pH [17]. However, the different behaviour of peptides **I** and **II** is rather unexpected as in both cases the charges are separated by three hydrophobic, non-charged amino-acid residues. Preliminary results indicate that a higher aggregation tendency of peptide **I** is responsible for the observed behaviour. More detailed studies on the relationship between helix formation and aggregation will shed light on these interesting findings [20].

When the pH is reduced to 1, a dramatic change in the conformational properties of peptide **I** is observed. As can be seen from *Fig. 4,* the CD spectrum of the peptide with fully protonated Glu side-chains is typical for a helical conformation, exhibiting two negative *Cotton* effects at 208 nm and 223 nm *(Table)*. In the absence of electrostatic repulsions, the helix-forming potential of peptide **I** overcomes any disturbing effects arising from aggregation, as postulated for **I** at pH 7. As expected from studies on

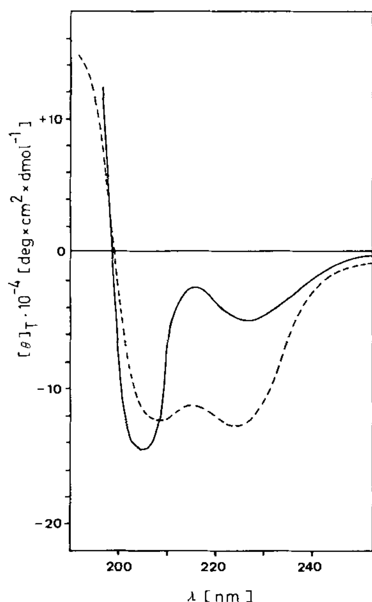


Fig. 4. *CD spectra of peptide* **I** *in H_2O at pH 7 (——) and pH 1 (---)*

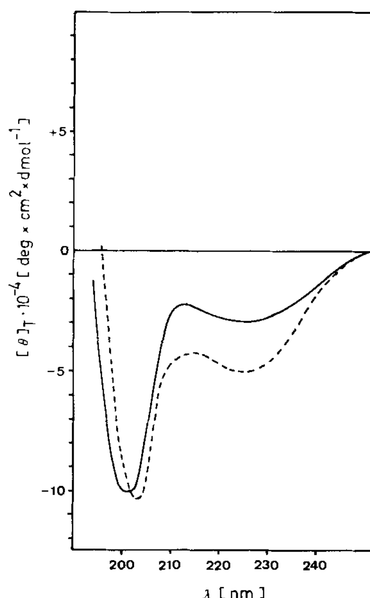


Fig. 5. *CD spectra of peptide* **III** *in H_2O at pH 7 (——) and pH 2 (---)*

oligo-Glu [17] [21], peptide **III** also exhibits a conformational transition after protonation of the Glu side-chains *(Fig. 5)*. A red shift of the π-π^* transition from 201 nm to 203 nm is observed, and R increases from 0.29 to 0.45. In view of the considerations above, the onset of a 3_{10}-helical structure appears to be very likely for peptide **III**. In peptide **II**, a red shift of the π-π^* transition to 207 nm and an increase of R from 0.87 to 1.03 is observed when changing the pH from 7 to 1 *(Fig. 3, Table)*. This points to a higher degree of helicity of the peptide in the side-chain-protonated state.

Conclusions. – The conformational investigations described above show that peptides **I–III** are able to adopt helical structures with amphiphilic character in solution, depending on the specific experimental conditions. From an analysis of the conformational behaviour with respect to the primary sequence of the peptides, some general guidelines for the construction of tailor-made amphiphilic helices can be derived: *a*) In peptides of the general sequence $(A\text{-}A\text{-}A\text{-}C)_n$, with A = nonpolar residue C = charged polar residue, and $n \geq 3$, the displacement of one apolar residue (*e.g.* Ala) by Aib results in a significant enhancement of the helix-forming potential of the peptide.

b) Peptide sequences of the type $(P\text{-}X\text{-}A\text{-}Aib)_n$, with A = nonpolar residue, X = uncharged polar residue or nonpolar residue, P = uncharged polar residue, and $n \geq 3$, form stable amphiphilic helices over a large scale of experimental conditions.

The unusual behaviour of amphiphilic peptides with helix-forming potential may originate in the propensity of forming defined aggregates in solution. These properties will be the subject of a forthcoming communication [20].

Experimental Part

Synthesis of the Peptides. All amino acids (except Gly) are in the L-configuration. The solvents and reagents used were of the highest purity available, and, in the case of liquids, they were freshly distilled and dried over molecular sieves. Polyethyleneglycol monomethyl ether (HO-PEGM; $M_r = 5 \cdot 10^3$) was a product of *Union Carbide*, U.S.A. The conversion of HO-PEGM into 'amino-PEGM' (H_2N-PEGM) was described earlier [22].

The synthesis of PEGM-peptides was performed according to the general procedures of the liquid-phase method [14]; dipeptides were used as coupling units in most cases instead of single amino acids. Each coupling step was tested for completion by ninhydrin and fluorescamine reactions [23]. The intermediate and final products were controlled for purity by amino-acid analysis, TLC, and enantiomer separations by GLC.

Peptides **I** *and* **III**. The base-labile Fmoc protecting group [24] was used for N_α-protection and the side-chain of Glu was protected as acid-labile *t*-butyl ester. Removal of the Fmoc group was achieved by treatment with 20% piperidine/DMF (30 min, r.t.). At the end of the synthesis, the side-chain-protecting groups were removed by 70% CF_3COOH/CH_2Cl_2 in the presence of anisole and ethane-1,2-thiol. Chain-elongation was performed by DCC/HOBt couplings of the dipeptides Fmoc-Glu(OBu^t)-Aib-OH, Fmoc-Ala-Aib-OH, and Fmoc-Glu(OBu^t)-Glu(OBu^t)-OH; for peptide **I**, Gly, Glu-2, and Pro were incorporated as single amino-acid derivatives. The dipeptides were synthesized by the hydroxysuccinimide ester/trimethylsilyl ester method [25], and their purity was checked by TLC, NMR, IR, and HPLC. Fmoc-amino acids and their hydroxysuccinimide esters were prepared by standard procedures [26] [27]. In the synthesis of peptide **I**, the very acid-labile anchoring group derived from 4-hydroxymethyl-3-methoxyphenoxyacetic acid [28] was used as a reversible link between peptide and polymer chain. The OH groups of the anchor were esterified with Fmoc-Gly in the presence of DCC and HOBt and further chain-elongation was achieved as mentioned above. At the end of the synthesis, the peptide was split off from the polymeric support by treatment with 70% CF_3COOH/CH_2Cl_2 with simultaneous removal of the side-chain-protecting groups. The peptide was separated from PEGM and purified by gel chromatography on *Sephadex LH 20* (MeOH) followed by ion-exchange chromatography on *DEAE Sephadex A 25* (pH 8, ammonium acetate buffer). In a last step, the peptide was subjected to ultrafiltration to remove any residual salts.

Peptide **II**. In this case, the acid-labile Boc protecting group was used for N_α-protection and the side-chain of Glu was protected as benzyl ester. The Boc group was removed by treatment with 1.2N HCl/AcOH (30 min) and the side-chain-protecting groups by catalytic hydrogenation over Pd/C in MeOH. As in the case of **I** and **III**, chain-elongation was mainly achieved by dipeptide couplings using DCC/HOBt activation. Boc-Glu(OBzl)-Ala-OH was synthesized *via* Boc-Glu(OBzl)-OSu in aq. Na_2CO_3/dioxane [29], whereas Boc-Ala-Aib-OH was prepared by the hydroxysuccinimide ester/trimethylsilyl ester method [25]. Both dipeptides were checked for purity by TLC, NMR, IR, and elementary analysis. Boc-amino acids and their hydroxysuccinimide esters were prepared by standard procedures [30] [27].

CD Measurements. CD spectra were recorded on a *JASCO J 500-A* and a *Yobin Yvon Mark V* circular dichrometer. Quartz cells of the path lengths 0.01 cm, 0.05 cm, and 0.1 cm were used, the concentration of the solns. being in the range from 0.2 to 1.2 mg peptide/ml. Ellipticities are expressed as total molar ellipticities $[\theta]_T$.

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