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Short model peptides having a high α-helical tendency: design and solution properties

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Secondary structure is not typically observed for small peptides in solution. Several of the properties of α-helical peptides are known which lead to the stabilization of the structure. The utilization of all the known factors important for α-helical stabilization in the design of model α-helical peptides (MAP) is reported. The peptides are based on the repeating eleven amino acid sequence, Glu-Leu-Leu-Glu-Lys-Leu-Leu-Glu-Lys-Leu-Lys (MAP_{1-11}). The CD spectra of these peptides give evidence for more α-helical content than has been reported for any short peptide (<18 amino acids) to date. This α-helical tendency does not require the presence of lipid or reduced temperature. For instance, Suc-[Trp^9]MAP_{9-3} amide (5), a seventeen amino acid peptide has 100% and 80% α-helical contents at 1.7×10^{-4} M and 1.7×10^{-5} M, respectively. Suc-[Trp^9]MAP_{2-11} amide (3), merely ten amino acids in length, is 51% α-helical at 1.7×10^{-4} M in 0.1 M phosphate buffer at room temperature. In the presence of lipid or trifluoroethanol, the α-helical content of these peptides is increased. This series of peptides demonstrates the complimentarity of various secondary structure design principles and the extent to which structure can be induced in small linear peptides.

Amphipathic α-helix; Model peptide

1. INTRODUCTION

Many efforts have been directed at the design of model peptides that form stable α-helices in the presence of lipid [1–8] or alone in aqueous solution [9]. Such peptides are valuable in understanding the forces that govern secondary structure formation and the mode of interaction of lipid-binding peptides with membranes. Short peptides (<18 amino aids) that have a high amount of α-helical content (>80%) in aqueous solution at room temperature in the absence of lipid have not yet been reported. Also, the minimum length of peptides having high α-helical tendency and lipid-binding affinity has not been established. A lower limit of as few as 13 residues has been suggested for the formation of a helical structure, based on work with ribonuclease A [1–13] at 0°C [10]. Typically, small peptides that have been reported to date have high amounts of α-helical content only at reduced temperature or in the presence of lipid [1–8]. In the present studies, a model amphipathic α-helical peptide (MAP) sequence was designed incorporating several means of α-helical stabilization and amphipathic analysis in order to produce a series of short, highly α-helical peptides that have a simple and regular sequence and also have the ability to bind lipid.

The first criterion in the design of MAP was that amino acids had the highest probability of being found in an α-helical peptide and a low probability of β-sheet or turn structures. Leu, Lys and Glu were chosen on this basis from their corresponding P_a, P_β and P_t values of Chou and Fasman [11]. Se-

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Abbreviations: Boc, *t*-butoxycarbonyl; Bzl, benzyl; CHO, formyl; 2-ClZ, 2-chlorobenzyloxycarbonyl; DCM, dichloromethane; DMF, dimethylformamide; FAB-MS, fast atom bombardment mass spectrometry; HPLC, high-performance liquid chromatography; MAP, model α-helical peptide

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cond, the Lys residues were positioned on the lateral faces of the α-helix and the Glu residues opposite the Leu residues (fig.2). This was done to provide the optimal charge distribution for lipid interaction [3]. Such a regular distribution of functional groups might also promote aggregation. Third, the size of the lipophilic face of the amphipathic α-helix probably has a great effect on the nature of the peptide-lipid interaction, thus, the lipophilic region was modelled after a well characterized lipid-associating peptide, 18As [3]. Fourth, an attempt was made to position the Glu and Lys residues in order to take advantage of potential helix stabilization through the formation of salt bridges. Work on model peptides by Marqusee and Baldwin [9] indicate that salt bridges can form in α-helical peptides between Glu and Lys residues that reside at positions i and $i+4$. Salt bridges apparently do not form between such residues when they are located at positions i and $i+3$ in the peptide [9]. Extensive use of this form of stabilization was not compatible with the second and third considerations stated above. Most of the spacings between the Glu and Lys residues in the model peptide were i to $i+3$ although one potential i to $i+4$ salt bridge exists in the sequence. Finally, helix dipole stabilization [12] was considered and the peptides incorporate desamino N-termini and C-terminal amides to neutralize the detrimental terminal charges. In addition, an effort was made to arrange the distribution of Glu and Lys residues in the sequence such that the Glu residues were closer to the N-terminus and the Lys residues were closer to the C-terminus. Fig.1 shows the peptides that were synthesized. They are based on an eleven amino acid sequence, Glu-Leu-Leu-Glu-Lys-Leu-Leu-Glu-Lys-Leu-Lys, designated as MAP monomer or MAP_{1-11}, which was the product of the above criteria.

2. EXPERIMENTAL SECTION

2.1. *Peptide synthesis*

The peptides were synthesized by solid-phase methods on an Applied Biosystems model 430A peptide synthesizer using protocols supplied by the manufacturer. The N^{α}-*t*-Boc-protected amino acids (Peptides Intl) were double coupled as their symmetrical anhydrides, first with dimethylformamide (DMF), then with dichloromethane (DCM) as a solvent, to *p*-methylbenzhydrylamine resin (0.65 mmol amine/g resin; Peptides Intl). The side chain protection used was Glu(Bzl), Lys(2-ClZ) and Trp(CHO). The N-terminal succinyl group was incorporated with the free N^{α}-amine peptide still attached to the resin using succinic anhydride in DMF/DCM (1 : 1). Deprotection and resin cleavage of the peptides was accomplished with HF/anisole/ethanedithiol (85 : 10 : 5) at -5°C (salt-ice bath) for 40 min. Upon removal of the HF in vacuo the peptides were precipitated with ether and extracted from the resin with 30% acetic acid. The extract was lyophilized and the residue purified by reverse-phase high-performance liquid chromatography (HPLC) using a Dynamax C18 column (40 × 250 mm) and acetonitrile in 0.1% aqueous trifluoroacetic acaid as an eluant. The purity and identity of the peptides was assessed by analytical HPLC, amino acid analysis and fast-atom bombardment mass spectrometry (table 1).

2.2. *Circular dichroic spectroscopy*

Circular dichroic spectra of peptide samples (1.7×10^{-4} and 1.7×10^{-5} M in 0.1 M phosphate buffer, pH 7.4) in 1 mm cuvettes (Hellma) were obtained at 25°C on a Jasco J-500A spectro-polarimeter with a 2 nm slit width. The CD of buffer alone was subtracted from the CD of the sample after each scan. For purposes of the calculation of the molar ellipticities the N-terminal succinyl group was considered to be a residue. A total of nine scans were averaged (fig.3). By the method of Morrisett et al. [13] the molar ellipticities at 222 nm were used to obtain estimates of the secondary structure of the peptides in solution (table 2).

3. RESULTS AND DISCUSSION

Peptides **1–6** (fig.1) were synthesized by solid-phase techniques. They represent different lengths and phases of the MAP sequence. The term 'phase' refers to peptides beginning at different points in the repeating eleven amino acid sequence. The N-termini are succinylated and the C-termini are amides to remove the positively charged terminal groups. A Trp residue replaces one of the Lys residues in all but the shortest peptide. This residue was incorporated as a fluorescent reporter group. Table 1 gives the analytical information on the peptides. All of the peptides have good water solubility. Circular dichroic (CD) spectra were run on the peptides in 0.1 M phosphate buffer at 25°C (fig.3). These peptides exhibited the greatest amount of α-helical content yet reported for peptides of this length under these conditions. Compound **5**, a seventeen amino acid peptide, gave a spectrum at 1.7×10^{-4} M that was indistinguishable from the polylysine spectrum that is typically used as a standard for defining 100% α-helical conformation (table 2). The tendency to form α-helix is very strong as demonstrated by the presence of a large amount of α-helical content (51% at 1.7×10^{-4} M) observed for the decapep-

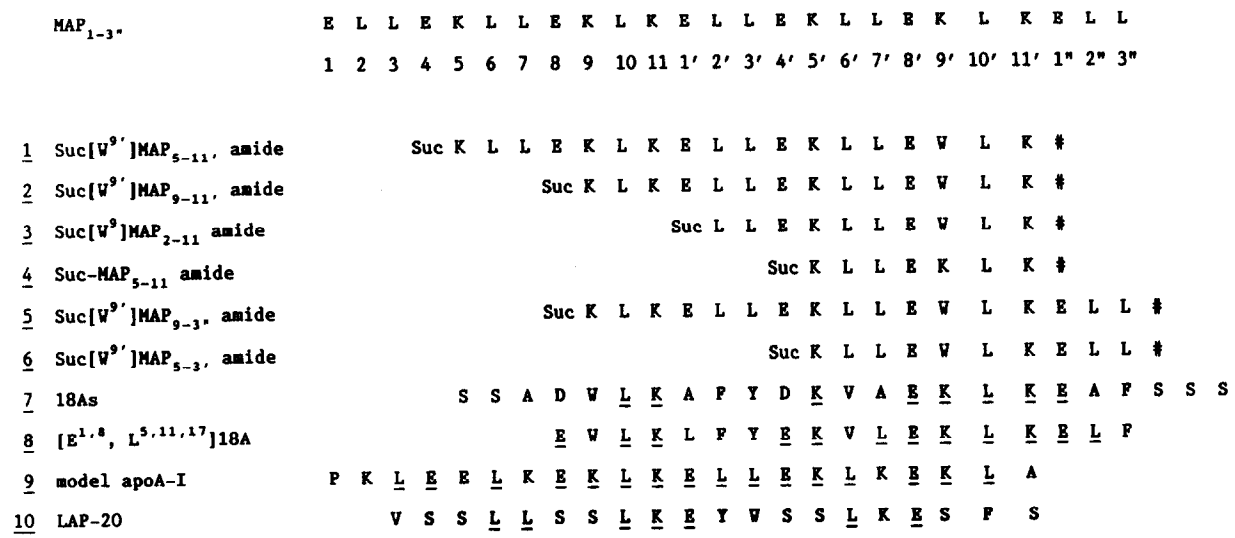

Fig.1. Amino acid sequences of MAP, analogs **1–6**, 18As (**7**; [3]), an 18As analog (**8**; [5]), a model apolipoprotein A-I peptide (**9**; [1]) and a model lipid associating peptide (LAP-20, **10**; [7]). Regions homologous to the MAP sequence in the latter four peptides are indicated by underlining of the residues. MAP is the oligomer X-Y_n-Z where X is a C-terminal fragment of the eleven amino acid MAP monomer, Y is the MAP monomer repeated *n* times and Z is an N-terminal fragment of the MAP monomer. Numbering begins with the first MAP monomer fragment. Residues in the subsequent MAP repeats are designated with the sequential addition of a prime symbol to the MAP monomer residue number. Compound **1** is N^{α}-succinyl-[$Trp^{9'}$]-$MAP_{5-11'}$ amide. Compound **2** is N^{α}-succinyl-[$Trp^{9'}$]$MAP_{9-11'}$ amide. Compound **3** is N^{α}-succinyl-[Trp^{9}]MAP_{2-11} amide. Compound **4** is N^{α}-succinyl-MAP_{5-11} amide. Compound **5** is N^{α}-succinyl-[$Trp^{9'}$]$MAP_{9-3'}$ amide. Compound **6** is N^{α}-succinyl-[Trp^{9}]$MAP_{5-3'}$.

tide **3**. The α-helical content observed was concentration dependent, particularly in the cases of the decapeptides suggesting that aggregation of the peptides lends added stability to the helical conformation. Taking this into account, the helical content observed for **5** is much greater than that observed for even the 74 amino acid, model 4-helix bundle protein, α_4, of DeGrado ($[\theta]_{222} = -21\,000$) [19]. Gel filtration of **5** and **6** at varying concentrations also gives evidence of greater aggregation with increasing concentration, but the formation of distinct 4-helix bundles was not observed (not shown). Even at 1.7×10^{-5} M, **1**, **2** and **5** exhibited 51, 45 and 80% α-helical contents, respectively. The dramatic helix-forming tendencies of the MAP sequence demonstrates that the various elements in the design of these peptides are complementary to one another.

Table 1

Amino acid analysis and FAB-MS for **1–6**

	Amino acid analysis[a]			Calcd molecular mass	FAB-MS[b] $(M+H)^+$
	Glx	Leu	Lys		
1	4.12 (4)	8.03 (8)	4.85 (5)	2364	2364
2	3.06 (3)	6.01 (6)	3.93 (4)	1881	1883
3	1.97 (2)	5.09 (5)	1.94 (2)	1383	1384
4	0.89 (1)	3.20 (3)	2.91 (3)	970	971
5	4.09 (4)	7.85 (8)	4.06 (4)	2236	2236
6	2.03 (2)	4.98 (5)	2.00 (2)	1383	1383

[a] 6 N HCl: 24 h or 48 h at 106°C
[b] $(M+H)^+ \pm 1$ mU

Within a series having the same phase of the MAP sequence, **1–4**, α-helical content decreased with the length of the peptide. When comparing peptides of comparable length but different phasing (e.g. **1** vs **5**) the α-helical content was not predictable and was apparently due to either overall hydrophobicity (**1** contains one more hydrophilic residue than **5**) or to a yet unparameterized form of stabilization (e.g. some measure of the ability of a peptide to self aggregate), since it did not correlate with the number of i to $i+4$ salt bridges, helix dipole stabilization, the per residue helical moment [14], the Chou-Fasman [11] $\langle P_{\alpha} \rangle$ or the length of the peptide (table 2). Different phases of the sequence having

Table 2

Physical data and calculations for **1** to **10**

Compound no.	Chou-Fasman[a] $\langle P_\alpha \rangle$ $\langle P_\beta \rangle$ $\langle P_t \rangle$	Eisenberg[b] per residue moment	No. amino acids	No. i to $i+4$ salt bridges	Overall charge pH 7	Morrisett et al.[c] analysis of CD spectra (25°C)							
						In 0.1 M phosphate				With DMPC		80% TFE	
						1.7×10^{-4} M		1.7×10^{-5} M		1.7×10^{-5} M		1.7×10^{-5} M	
						$[\theta]_{222} + 10^3$	%α	$[\theta]_{222} + 10^3$	%α	$[\theta]_{222} + 10^3$	%α	$[\theta]_{222} + 10^3$	%α
1	1.26 0.94 0.80	0.442 @ 103°	18	1	0	−28.1	76	−19.8	51	−19.5	50	−19.8	51
2	1.25 0.95 0.80	0.414 @ 104°	14	1	0	−25.7	69	−17.9	45	−20.8	54	−17.9	45
3	1.25 1.01 0.74	0.422 @ 106°	10	1	−1	−19.9	51	−5.6	8	−12.2	28	−7.4	13
4	1.23 0.93 0.79	0.547 @ 117°	7	0	+1	−3.0	0	−0.1	0	+13.4	[d]	+17.2	[d]
5	1.26 0.95 0.74	0.395 @ 101°	17	1	−1	−36.7	100	−29.5	80	−38.3	100	−32.1	88
6	1.25 1.01 0.74	0.419 @ 102°	10	0	−1	−15.6	38	0.7	0	−18.7	48	−8.6	17
7	1.08 0.91 0.97	0.264 @ 95°	24	1	0	–	–	−3.1[e,f]	0	−8.6[f]	17	−12.0[f]	27
8	1.21 1.01 0.76	0.352 @ 94°	18	1	0	–	–	−6.2[g]	10	–	–	–	–
9	1.26 0.81 0.81	0.270 @ 99°	22	2	+1	−16.5[h]	41	−8.7[h]	17	–	–	–	–
10	1.02 0.97 1.02	0.276 @ 102°	20	0	0	–	–	−8[i]	15	−31[i]	85	–	–

[a] [11]
[b] [14]
[c] [13] % α-helix = $-(([\theta]_{222} + 3000)/33000) \times 100$
[d] Under these conditions the peptide exhibited a CD spectrum indicative of β-turn conformation
[e] [3] in 0.02 M sodium phosphate, pH 7.4
[f] [8]
[g] [5] in 0.01 M sodium phosphate, pH 7.0, 150 mM NaCl, 25°C, at 5.7×10^{-5} M
[h] [1] in 0.01 M phosphate buffer, pH 7.0, at concentrations of 1.7×10^{-4} and 3×10^{-6} M
[i] [7] 6.2×10^{-5} M at 12°C

a similar length, as exemplified by compounds **3** and **6**, behaved similarly under the conditions that were examined, but **6** had greater α-helical content in the presence of lipid than **3** (48% vs 28%). Both have almost identical Chou-Fasman and helical moment parameters. Compound **3** has the potential for a salt bridge between the N-terminal succinyl group and Lys-5 and the Glu and Lys residues of this peptide are better distributed for helix dipole stabilization than they are in **6**. The degree to which the various elements of helix stabilization affect lipid interaction, aggregation and helix-forming ability of peptides cannot be assessed using these data.

In the presence of lipid or 80% trifluoroethanol in 0.1 M phosphate buffer most of the peptides exhibited enhanced α-helical content relative to that seen in phosphate buffer alone. Compound **4**, which contains only seven amino acids, exhibits no α-helical content alone or in the presence of lipid or 80% trifluoroethanol. It does appear to have a high degree of β-turn content, based on comparison to standard spectra [15], which is enhanced

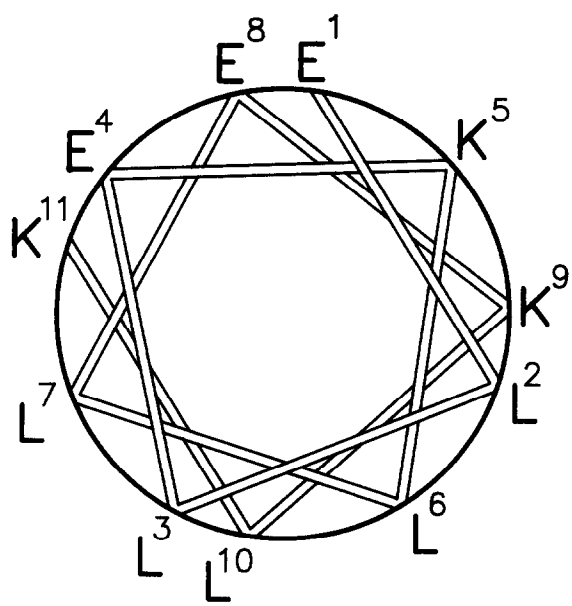

Fig.2. Edmundson wheel diagram [20] of **1**.

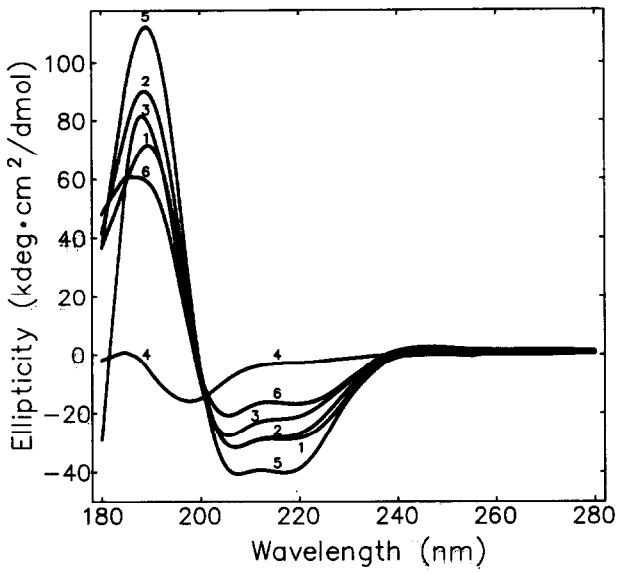

Fig.3. Circular dichroism spectra of **1**–**6**.

by the presence of lipid or 80% trifluoroethanol. This degree of structure for such a short linear peptide containing no unusually hindered amino acids is remarkable. No β-turns are indicated by Chou-Fasman calculations. The sequence Lys-Asp, but not Orn-Asp or Asp-Lys, has been reported to lead to the stabilization of β-turn structures via side chain interaction [16]. Peptide **4** contains a Glu-Lys sequence. We are not aware of any evidence that this sequence could also stabilize a β-turn conformation, but it is a possibility that cannot be discounted at this time. Therefore, the driving force for the transition from α-helical to β-turn structure upon shortening of the peptide is unclear. The enhancement of structure by lipid suggests the importance of amphipathic structure in the overall conformation.

In conclusion, the understanding of the factors that influence the secondary structure of peptides has been greatly advanced in recent years by the study of model peptides [1–10,12]. Utilization of the elements leading to structural stability allows the enhancement of secondary structure in model peptides, as demonstrated in this paper. Thus, studies into the basic mechanisms of structural formation in peptides and proteins may soon prove to be most valuable in the design of synthetic proteins [18,19] and pharmaceutically useful materials.

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