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Lactam Bridge Stabilization of α-Helical Peptides: Ring Size, Orientation and Positional Effects

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Abstract: A series of 14 residue amphipathic α-helical peptides, in which the sidechains of glutamic acid and lysine have been covalently joined, was synthesized in order to determine the effect of spacing, position and orientation of these lactam bridges. It was found that although an $(i, i+3)$ spacing would position the lactam bridge on the same face of the helix, these lactams with 18-member rings were actually helix-destabilizing regardless of position or location. On the other hand, $(i, i+4)$ lactams with 21-member rings were helix-stabilizing but this was dependent on orientation. Glutamic acid-lysine lactams increased the helical content of the peptide when compared with their linear homologue in benign conditions (50 mM KH_2PO_4, 100 mM KCl, pH 7). Two Glu-Lys $(i, i+4)$ lactams located at the N- and C-termini gave rise to a peptide with greater than 99% helical content in benign conditions. Peptides with Lys-Glu oriented lactams were random structures in benign conditions but in the presence of 50% TFE could be induced into a helical conformation. The stability of the single-stranded α-helices, as measured by thermal denaturations in 25% TFE indicated that Glu-Lys oriented lactam bridges stabilized the helical conformation relative to the linear unbridged peptide. One Glu-Lys lactam in the middle of the peptide was more effective at stabilizing helical structure than two Glu-Lys lactams positioned one at each end of the molecule. The lactams with the Lys-Glu orientation were destabilizing relative to the unbridged peptide. This study demonstrates that correct orientation and position of a lactam bridge is critical in order to design peptides with high helical content in aqueous media.

Keywords: Lactam bridges; α-helical stability; cyclization

Abbreviations

AcCN, acetonitrile; BOP, benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate; CD, circular dichroism; 2-ClZ, 2-chlorobenzyloxycarbonyl; GRH, growth hormone releasing factor; HFIP, 1,1,1,3,3,3-hexafluoroisopropanol; HBTU, 2-(1H-benzotriazolyl)-1,1,3,3-tetramethyluronium hexafluorophosphate; NMM, *N*-methylmorpholine; NMP, N-methylpyrrolidinone; OFm, 9-fluorenylmethyl; RPC, reversed-phase chromatography; TFE, 2,2,2-trifluoroethanol.

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INTRODUCTION

α-Helices found in proteins are typically 10 to 12 amino acids in length [1], but peptides of this size are generally devoid of structure and assume multiple random-like conformations. Because of this, a number of strategies have been employed to induce and stabilize helical structure in *de novo* designed peptides. Marqusee and Baldwin [2] demonstrated the efficacy of Glu-Lys salt bridges spaced $(i, i+4)$ in stabilizing an α-helical conformation in short alanine-based peptides. Their work showed that a 16 residue peptide with 4 Glu-Lys salt bridges was 80% helical at 1°C. Scholtz *et al.* [3] investigated the energetics of Glu-Lys ion pairs in a helical peptide and concluded that $(i, i+3)$ and $(i, i+4)$ interactions are helix-stabilizing and similar in strength to each

other regardless of their orientation. Other approaches have stabilized helical structure by covalent interactions between properly oriented sidechains. Ghadiri and co-workers employed peptide–metal complexes in which histidines were ligated to a number of transition metals and the resulting peptides had substantial helical character in aqueous solutions [4, 5].

In an alternative approach, Felix *et al.* [6] and Madison *et al.* [7] described the synthesis of analogues of human growth hormone releasing factor (GRF) in which a lactam bridge was formed between aspartic acid 8 and lysine 12 of the peptide giving a 20-member ring. This lactam bridge was formed on the resin using a combination of OFm and Fmoc deprotection followed by cyclization with BOP [8]. This covalent link was found not only to enhance the bioactive helical conformation of this peptide but led to increased biological potency [6]. Their work, showed that lactam bridges that formed 21- and 20-member rings were more helical than 19-member rings, which destabilize helical structure [9]. Similarly, Osapay and co-workers [10, 11] described the synthesis of an amphipathic peptide containing three Lys-Glu lactam bridges prepared by a combination of cyclization on a *p*-nitrobenzophenone oxime resin followed by segment condensation. However in aqueous solution this peptide adopted a discorded conformation but was highly helical in 50% TFE. This prompted us to re-evaluate the ability of lactam bridges to induce and stabilize on α-helical conformation in aqueous conditions. The experimental goal of this study was to determine the effects of spacing, location and orientation of lactam bridges in helix formation and stability of peptides. To this end, a series of lactam-bridged peptides were synthesized and conformationally characterized by CD. The ability of lactam bridges to impart and stabilize helical structure was directly compared to linear, non-cyclized peptides based on the same sequence.

MATERIALS AND METHODS

Peptide Synthesis and Purification

All peptides were prepared by solid-phase peptide synthesis using a benzhydrylamine-hydrochloride resin on a Labortec SP 640 peptide synthesizer. All amino acids were *N*-α-*t*-butyloxycarbonyl protected with lysine and glutamic acid sidechain functionalities protected as 2-ClZ and OBzl derivatives respectively. The sidechains of glutamic acid and lysine residues involved in lactam formation were protected as OFm and Fmoc derivatives respectively. A coupling protocol using a fivefold excess of HBTU/HOBt/amino acid/NMM (1:1:1:1.5) in NMP with a 5 min activation time was employed. Lactam bridges were formed on the resin by deprotection of the desired sidechains with 20% piperidine in NMP followed by cyclization with fivefold excess of HBTU/HOBt/NMM in NMP with 5% HFIP. To facilitate the intrachain reaction, substitution levels were maintained at or below 0.15 mmol of amino groups per gram of resin. Substitution levels were determined spectrophotometrically according to the method of Meienhofer *et al.* [12]. The peptides were cleaved from the resin by reaction with HF (10 ml/g resin) containing 10% anisole for 1 h at −5 to 0°C. The crude peptides were purified by reversed-phase high performance liquid chromatography (RPC) on a SynChropak RP-4 preparative C4 column (250 × 21.2 mm internal diameter, 6.5 μm particle size, 300 Å pore size) (SynChrom, Lafayette IN) with a linear AB gradient of 0.1% B/min with a flow rate of 5 ml/min, where solvent A is 0.05% trifluoroacetic acid in water and solvent B is 0.05% TFA in acetonitrile. For amino acid analysis, peptides were hydrolysed in 6 N HCl containing 0.1% phenol for 1 h at 160°C in sealed evacuated tubes. Amino acid analysis was performed on a Beckman model 6300 amino acid analyser (Beckman, San Ramon, CA). The correct primary ion molecular weights of the peptides were confirmed by plasma desorption time of flight mass spectroscopy on a BioIon-20 mass spectrometer (Uppsala, Sweden).

Circular Dichroism Measurements

Circular dichroism spectra were measured on a Jasco J-500C spectropolarimeter (Jasco, Easton, MD) equipped with a Jasco DP-500N data processor. A Lauda water bath (model RMS, Brinkmann Instruments, Rexdale, Ont.) was used to control the temperature of the cell. Constant N_2 flushing was employed. The instrument was routinely calibrated with an aqueous solution of *d*-10-(+)-camphorsulphonic acid at 290 nm. Ellipticity is reported as mean residue molar ellipticity ([θ]), in deg cm^2/dmol and calculated from the equation:

$$[\theta] = \theta_{obs}(\text{mrw})/10lc$$

where θ_{obs} is the ellipticity measured in degrees, mrw is the mean residue weight (molecular weight divided by the number of amino acid residues), c is the peptide concentration in grams per millilitre and l

is the optical path length of the cell in centimetres. CD spectra were the average of four scans obtained by collecting data at 0.1 nm intervals from 255 to 190 nm. Peptide concentrations were determined by amino acid analysis.

RESULTS

Peptide Design

The first five sequences in Table I are based on the sequence:

Ac-EIEALKKEIEALKK-amide

which allows for the incorporation of lacatam bridges between the sidechains of glutamic acid and lysine residues at the N- or C-termini or in the middle of the peptide at ($i, i+3$) or ($i, i+4$) spacings. The repetitive nature of an α-helix (3.6 residues per turn) implies that lactam bridges formed between residues spaced ($i, i+3$) or ($i, i+4$) will fall on the same face of the helix. Where sequence changes were made to study different orientations of the lactam bridge (Glu-Lys *v.* Lys-Glu) the linear homologue was synthesized as a control. All peptides are 14 residues in length and contain an equal number of acidic and basic amino acids. Glutamic acid residues were located near the N-terminus and lysine residues near the C-terminus such that the interaction of these charged sidechains with the helix dipole would be attractive in nature and enhance helicity [13, 14]. In addition, the Ile and Leu residues are arranged in a 3,4 hydrophobic repeat in which they occupy positions *a* and *d* of the repeating heptad denoted *abcdefg*, characteristics of a two-stranded α-helical coiled-coil, in order to facilitate peptide dimerization through this hydrophobic face and hence to increase helical content [15–17]. Ile was selected for the position *a* and Leu for position *d* to provide maximal stability of the peptide dimer [18]. The N- and C-termini are capped with an acetyl and carboxamide functionalities respectively in order to avoid unfavourable helix–dipole interactions [13, 14].

Dependence of Helical Content on the Type of Lactam Bridge

The far-ultraviolet CD spectra with one lactam located in the interior of the sequence under benign conditions (50 mM KH_2PO_4, 100 mM KCl, pH 7) is shown in Figure 1. All spectra were measured at a peptide concentration of $750 \pm 30\,\mu M$ in order to avoid any concentration dependency effects. Peptides KE ($i, i+3$) and KE ($i, i+4$) show a significant drop in helical content in benign medium when compared with their linear 5 homologue (Table II). The linear peptide was approximately 61% helical whereas KE ($i, i+3$) and KE ($i, i+4$) contained 12 and 29% helical content as calculated by the method of Chen *et al.* [19] (Table II). Peptide KE ($i, i+3$) contained significant random structure (Figure 1) as characterized by the minima at 198 nm [20]. In 50% trifluoroethanol, a solvent which is known to promote helix formation in peptides with helical propensity [21–24], peptide KE ($i, i+3$) was 64% helical. However, under identical conditions linear 5 and KE ($i, i+4$) were 98% and 92% helical respectively and were characterized by a maximum at 192 nm (>60 000) and minima at 208

Table 1 Amino Acid Sequences of Lactam-bridged and Linear Peptides. Peptides with Similar Sequences are Grouped Together. Residues that Form the Lactam Bridge are Underlined in Bold Type

Peptide number	Sequence	Lactam position	No. of lactam bridges	Peptide name[a]
1	Ac-EIEALK**K**EI**E**ALKK-amide	Lys_7-Glu_{10}	1	KE ($i, i+3$)
2	Ac-EIEAL**K**KEI**E**ALKK-amide	Lys_6-Glu_{10}	1	KE ($i, i+4$)
3	Ac-EI**E**ALK**K**EI**E**ALK**K**-amide	Glu_3-Lys_6, Glu_{10}-Lys_{13}	2	2EK ($i, i+3$)
4	Ac-EI**E**ALK**K**EI**E**ALK**K**-amide	Glu_3-Lys_7, Glu_{10}-Lys_{14}	2	2EK ($i, i+4$)
5	Ac-EIEALKKEIEALKK-amide	–	0	Linear 5
6	Ac-EIEAL**E**K**E**EI**K**ALKK-amide	Glu_6-Lys_{10}	1	EK ($i, i+4$)
7	Ac-EIEALEKEIKALKK-amide	–	0	Linear 7
8	Ac-EI**K**ALK**E**EI**K**ALK**E**-amide	Lys_3-Glu_7, Lys_{10}-Glu_{14}	2	2KE ($i, i+4$)
9	Ac-EIKALKEEIKALKE-amide	–	0	Linear 9
10	Ac-EIQALKK(Ac)EIQALKK(Ac)-amide	–	0	Linear 10

[a]The lactam bridge designation contains the sequence of the two residues involved from the N-terminal of the peptide in one letter code which gives the orientation of bridge and the bridge type as either i to $i+3$ or i to $i+4$. Peptides with two lactam bridges are designated by the number 2 preceding the sequence. Peptides with no lactam bridges are denoted as linear followed by the peptide number.

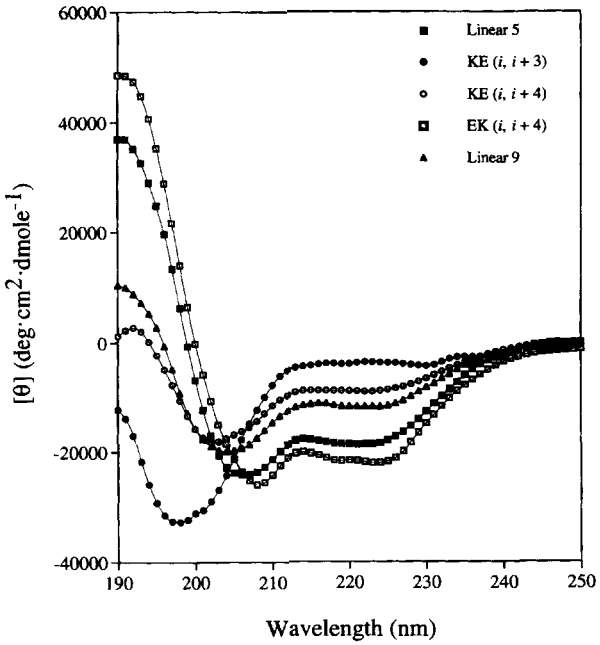

Figure 1 Circular dichroism spectra of peptides with one lactam located in the middle of the peptide and their linear homologues in benign medium at 20 °C. Peptide designations and amino acid sequences are shown in Table 1. Peptide concentrations are 750 ± 30 μM.

and 220 nm. Surprisingly, peptide EK ($i, i+4$) was considerably more helical in benign conditions than peptide KE ($i, i+4$) (71% *v.* 29%) and this increase in helicity cannot be attributed to sequence effects alone. Since both peptides have essentially the same helical content in 50% TFE (>90% helicity), it is likely that the orientation of the lactam bridge in peptide KE ($i, i+4$) (Lys to Glu) may have a destabilizing interaction between the lactam carbonyl and main-chain atoms of the helix. The amount of helix stabilization imparted by the EK ($i, i+4$) lactam (−10 100, $\Delta[\theta]_{222}$ (linear–lactam), Table 2) is comparable in magnitude to the amount of helix destabilization that results from a KE ($i, i+4$) lactam (−9600).

Figure 2 shows the CD spectra of peptides with lactams located at the N- and C-termini. Peptide 2EK ($i,i+3$) has a similar CD profile to peptide KE ($i, i+3$) and the same amount of helical content (12%, Table II). However, the amount of helical structure induced by 50% TFE is significantly less for peptide 2EK ($i,i+3$) compared with KE ($i, i+3$) ($\Delta[\theta]_{222}$ of 6100 *v.* 16 250). These results suggest that the ability of ($i,i+3$) lactams to induce helical structure in benign medium is quite limited. Similarly, peptide 2KE ($i, i+4$) and peptide KE ($i, i+4$) have nearly identical CD spectra and helical content under benign conditions (27 and 29% respectively), and in the presence of 50% TFE the helical content rises to greater than 90% (Table 2). In benign conditions, the minimum for 2KE ($i, i+4$) was shifted slightly from 202 to 201 nm, suggesting that the slight drop in $[\theta]_{222}$ value is a result of an increase in random structure. Surprisingly, two Glu-Lys ($i, i+4$) lactams at the ends of the peptide result in a peptide, 2EK ($i, i+4$), that is 99% helical under benign conditions. This peptide is

Table 2 Circular Dichroism Results of Lactam Bridges and Linear Peptides. Peptides with Similar Sequences are Grouped Together

Peptide	$[\theta]_{222}$(deg cm²/dmol)[a] Benign	50% TFE	$\Delta[\theta]_{222}$[b] (benign-TFE)	$\Delta[\theta]_{222}$[c] (Linear-lactam) benign	Helix content (%)[d] Benign	50% TFE
KE i (i+3)	−3600	−19 850	16 250	−15 000	12	64
KE i (i+4)	−9000	−28 400	19 400	−9600	29	92
2EK i (i+3)	−3800	−9900	6100	−14 800	12	32
2EK i (i+4)	−30 350	−32 150	1800	11 750	99[e]	105
Linear 5	−18 600	−30 000	11 400	-	61	98
2KE i (i+4)	−8300	−29 100	20 800	4650	27	95
Linear 9	−3650	−23 500	19 850	-	12	77
EK i (i+4)	−21 800	−29 200	7400	10 100	71[e]	95
Linear 9	−11 700	−28 900	17 200	-	38	94
Linear 10	−2900	−14 000	11 100	-	10	45

[a] Calculated molar ellipticity of the peptide at 222 nm.
[b] $\Delta[\theta]_{222}$ is the difference between the ellipticity at 222 nm in benign buffer and in 50% TFE.
[c] $\Delta[\theta]_{222}$ is the difference between the ellipticity at 222 nm of the linear peptide and lactam peptide of the same sequence.
[d] The % helical content was calculated from the ratio of the observed $[\theta]_{222}$ value divided by the predicted molar ellipticity. The predicted molar ellipticity (X^nH) was calculated from the equation $X^nH = X^\infty H(1-k/n)$, using a $[\theta]_{222}$ value of −37 400 for a helix of infinite length ($X^\infty H$), n equal to 14 and k the wavelength-dependent constant equal to 2.50 [19].
[e] Denotes the most favourable lactam bridge locations to induce helical structure in benign medium.

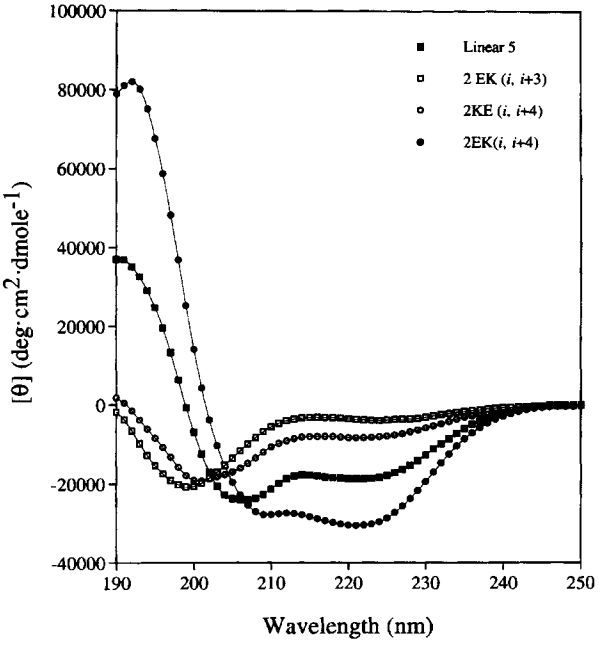

Figure 2 Circular dichroism spectra of peptides with two lactams located at the N- and C-termini and their linear homologues in benign medium at 20 °C. Peptide designations and amino acid sequences are shown in Table I. Peptide concentrations are 750 ± 30 μM.

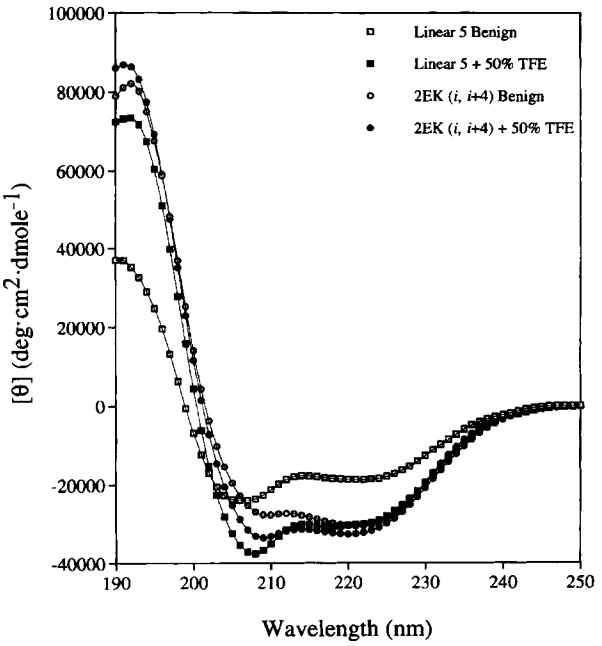

Figure 3 Circular dichroism spectra of 2EK (*i*, *i*+4) and linear 5 in benign medium and in the presence of 50% TFE. Peptide designations and amino acid sequences are shown in Table I. Peptide concentrations are 750 ± 30 μM.

characterized by a maximunm at 192 (82 000) and minima at 209 nm (−27 700) and 221 (−30 350). There is a slight increase in $[\theta]_{222}$ value from −30 350 to −32 150 on the addition of TFE. Since the peptide is essentially completely helical in benign medium, the observed increase in TFE may have little to do with helical structure but rather to the absorbance properties of the lactam-bridged peptide in TFE.

Figure 3 shows the CD spectra of 2EK (*i*, *i*+4) and its linear counterpart under benign conditions and in the presence of 50% TFE and illustrates the dramatic increase in helical content caused by properly located and oriented lactam bridges in benign conditions. The increase in helical content induced by two (*i*,*i*+4) lactams (11 750) is almost identical to the helicity induced by 50% TFE for the linear peptide (11 400), indicating the efficacy of lactam bridges in imparting helical structure. Another feature of the 2EK (*i*, *i*+4) spectrum is the change of the $\theta_{222}/\theta_{208}$ ratio in going from benign conditions (1.10) to 50% TFE (0.97). Such a feature has been observed with other synthetic peptides which undergo the transition from a two stranded coiled-coil to single-stranded α-helices [25–28]. In addition, the mean residue molar ellipticity at 222 nm shows a concentration dependency indicative of dimerization (Figure 4). The peptide remains greater than 95% helical over a concentration range of 6000–250 μM. Linear peptide 5 shows a concentration dependence over the entire range (6000–25 μM).

To determine whether the increased hydrophobi-

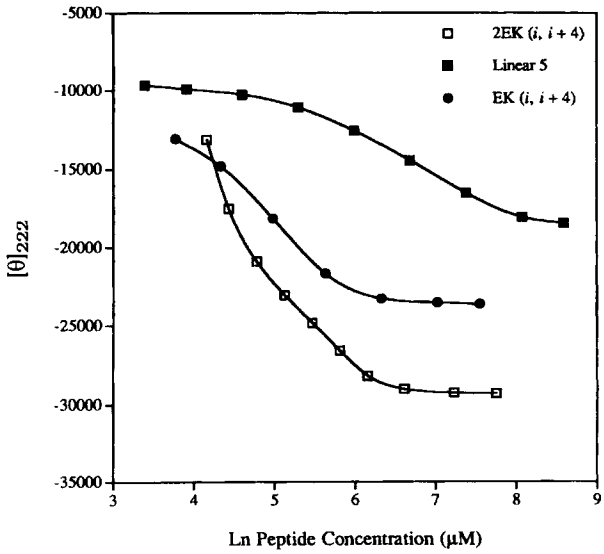

Figure 4 The effect of peptide concentration on the ellipticity at 222 nm at 20 °C for peptides 2EK (*i*, *i*+4), linear 5 and EK (*i*, *i*+4).

city associated with lactam formation played any role in enhancing helical content, linear peptide 10 was synthesized. It was thought that substitution of glutamic by glutamine and acetylation of the ε-amino group of lysine might resemble the hydrophobicity of the lactam bridges but without the constraints that cyclization places on the peptide. Under benign conditions, this peptide had a substantial amount of random structure with approximately 10% helical content (Figure 5, Table 2). In 50% TFE the helical content rose to 46%, suggesting that the hydrophobicity change upon formation of lactam bridges has little or no effect on stabilizing the helical content of peptide 2EK ($i, i+4$). The linear 5 peptide with 2EK salt bridges has 61% helical content in benign conditions and 98% α-helix in 50% TFE (Table 2, Figure 5). This also suggests that the two salt bridges which are present in linear 5 and absent in linear 10 offer considerable stability to the helix.

Dependence of Helical Stability on Lactam type

In order to determine the stabilizing effect of the various ($i, i+4$) lactams, thermal denaturations in the presence of 25% TFE were carried out. In addition to promoting α-helix formation in peptides with helical propensity, TFE has been shown to disrupt tertiary and quaternary interactions in peptides and proteins [24, 29]. Hence, thermal denaturations in the presence of TFE should be a measure of the stability of single stranded α-helices. The CD spectra of all five peptides showed an isodichroic point at 202 nm, which is consistent with the presence of just two conformations [26, 30]. Though all peptides have essentially the same helical content in the presence of TFE at 5°C, differences in helical content are observed as the temperature is increased (Figure 6). The stability of the peptides generally follows their helical content under benign conditions. The least stable peptides are the KE peptides with 2KE ($i, i+4$) being substantially less stable than KE ($i, i+4$) and both being less stable than the linear non-cyclized peptide. Obviously, there is destabilizing interaction involved in the KE lactam peptides and this interaction is additive.

On the other hand, the EK lactam peptides are substantially more stable than the linear peptide. Surprisingly, EK ($i, i+4$) is more stable than 2EK ($i,i+4$) even though under benign conditions two lactams are more effective than one lactam in promoting α-helical content in the peptide. At 80°C both peptides retained greater than 70% of their original α-helical content. The increase in α-helical content in peptide 2EK ($i, i+4$) most likely arises from the terminal lactam bridges locking the peptide into the desired conformation and negating end

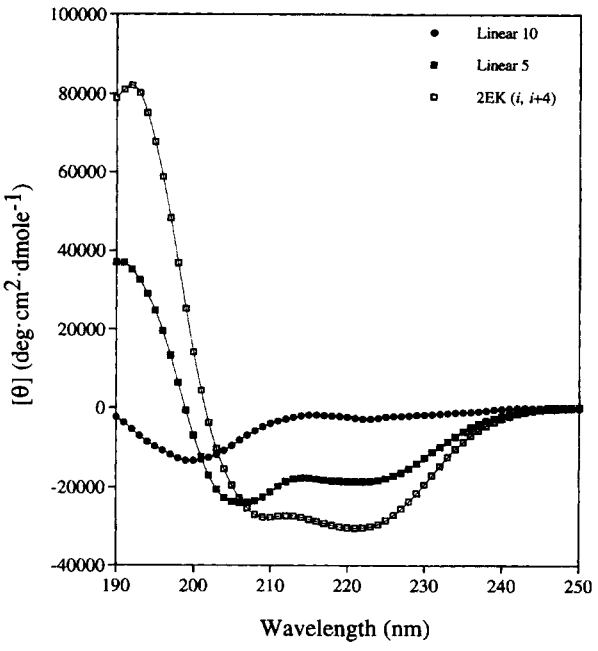

Figure 5 Circular dichroism spectra of 2EK ($i, i+4$), linear 5 and linear 10 in benign medium at 20°C. Peptide designations and amino acid sequences are shown in Table I. Peptide concentrations are 750 ± 30 μM.

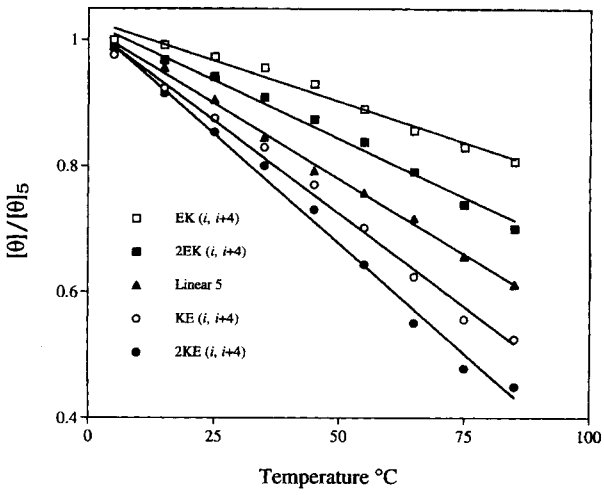

Figure 6 Thermal melting profiles of EK ($i, i+4$), 2EK ($i, i+4$), linear 5, KE ($i, i+4$) and 2KE ($i, i+4$) in 50 mM KH_2PO_4, 100 mM KCl buffer containing 25% TFE (v/v), pH 7. $[\theta]/[\theta]_5$ represents the ratio of the ellipticity at 222 nm at the indicated temperature to the ellipticity at 5°C.

effects. In the crystal structure of peptides of the coiled-coil region of GCN4, the N- and C-termini deviated from an α-helical conformation and were frayed [31]. Similar results were obtained from molecular dynamics simulation of model coiled-coils by Zhou *et al.* [27]. For a peptide of only 14 residues, such fraying would be thought to play a significant role in decreasing the α-helical content of the peptide. Peptide EK ($i, i+4$) would be expected to have frayed ends and this may account for the lesser amount of α-helical content in benign conditions. These results also suggest that placement of a lactam bridge in the middle of the peptide stabilizes the single-stranded α-helix more than at the ends of the molecule.

Peptides 2EK ($i, i+4$), EK ($i, i+4$) and linear 5 all show a dependence of helical content on peptide concentration (Figure 4), suggesting that dimerization plays a significant role in stabilizing helical content in addition to lactam bridges and salt bridges. The magnitude of the stability imparted by lactam bridges an be gleamed from these concentration dependency curves. Since linear 5 and 2EK ($i, i+4$) have identical sequences and identical residues at the hydrophobic interface, the difference in helical content at any concentration is due to lactam bridges enhancing helical structure to a greater extent than Glu-Lys salt bridges.

DISCUSSION

Comparison of ($i, i+3$) and ($i, i+4$) Lactams

One of the notable results from these experiments is the large difference in helical content in going from ($i, i+3$) to an ($i, i+4$) lactam bridge. Although both should lie on the same face of the helix, ($i, i+3$) lactams were not effective in stabilizing helical content in our model peptides. Our results mirror those of Marqusee and Baldwin [2] and Scholtz *et al.* [3] which showed that Glu-Lys salt bridges spaced ($i, i+4$) were more effective in inducing helical structure than ($i, i+3$)-spaced salt bridges. In fact, a considerable amount of random structure was imparted by the ($i, i+3$) lactams and this steric strain could not be alleviated even in the presence of the helix-inducing solvent, TFE. In the studies of Felix *et al.* [9] on lactam-bridged growth hormone-releasing factor, they concluded that for ($i, i+4$) lactams 20- and 21-member rings imparted significantly more α-helical structure than peptides with lactam rings of 19 atoms or less. The ($i, i+3$) lactams form a 18-member ring which may account for their inability to stabilize helical structure. Alternatively, the ($i, i+3$) spacing, which does not complete one full turn of the helix, may simply be geometrically unfavourable and thus impart steric strain into the peptide. The ($i, i+4$) lactam-bridged peptides of the present study form 21-member rings and consistent with previous results [6] these lactam bridges are capable of inducing helical structure.

Comparison of EK and KE $i, i+4$ Lactams

The second notable result was the dependence of helical content on the orientation of the ($i, i+4$) lactam bond. Lactam bridges oriented Lys to Glu were typically less helical than their linear homologues, although greater than 90% helical content could be induced by 50% TFE. A similar result was found for the sequence H–(KLKELKE)$_3$-OH in which ($i, i+4$) lactams between K_3 and E_7 of the repeating heptad adopted a disordered conformation in aqueous solution but was highly helical in 50% TFE [10, 11]. On the other hand, Glu to Lys lactams were more helical than their linear counterparts and this increase was similar in magnitude to that induced by 50% TFE. This orientational effect was also described by Marqusee and Baldwin [2] in their salt bridge-stabilized peptides, with Glu-Lys salt bridges affording greater helical content than Lys-Glu salt bridges, although the magnitude of the decrease was substantially less than in the case of lactam-stabilized peptides. This behaviour was attributed to a destabilizing interaction between the charged groups of the sidechains and the partial charges arising from the helix macrodipole. Such interactions cannot arise in lactam-bridged peptides as the amino and carboxyl groups are involved in an amide bond. A plausible explanation for this orientational effect with lactams may be due to the differences in length of the Glu and Lys sidechains which would result in the carbonyl groups of the lactam bridges being in different environments when the orientation is reversed. Preliminary modelling studies suggest that for KE lactams, the proximity of the carbonyl oxygen of the lactam bond falls within the van der Waals contact distance of the carbonyl oxygen of the ith peptide bond (Lys–Ala). The random structure found in benign conditions alleviates this disruptive interaction.

Positional Effects of EK ($i, i+4$) Lactams

Surprisingly, although peptide EK ($i, i+4$) is less helical than peptide 2EK ($i, i+4$) in benign conditions, thermal denaturations in 25% TFE suggest it

forms a more stable single-stranded α-helix. This suggests that stabilization of the central region of the helix is most important in preventing the unfolding of the monomeric helix. The increase in helical content of 2EK ($i, i+4$) may simply be a result of locking the peptide into a helical conformation and keeping the ends of the peptide from fraying. End effects should decrease the helical content of peptides substantially. The amount of helix content present in a peptide should play a role in the ability of a peptide to dimerize. In the case of 2EK ($i, i+4$), by locking the ends of the peptide into a helical structure, it would be expected that upon dimer formation the hydrophobic interaction would be maximized and hence the concentration dependency would be shifted to a lower concentration. However, this shift is not seen and in fact 2EK ($i, i+4$) and EK ($i, i+4$) have similar concentraton ranges over which they maintain their maximal helical content. The fact that increased helical content does not translate to a more stable monomeric helix or dimeric species may be related to the loss of conformational entropy at the ends of the molecule. By placing a lactam bridge in the middle of the peptide, which is a less dynamic part of the molecule, the loss of conformational entropy would be considerably less. Thus one cannot equate helical content with helical stability.

Our study of lactam-bridged peptides clearly shows the importance of lactam orientation in inducing and stabilizing helical content of small peptides. In addition, this study reaffirms the value of lactam bridges as a method of stabilizing an α-helical conformation in peptides.

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