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The conformational preferences of γ-lactam and its role in constraining peptide structure

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SUMMARY

The conformational constraints imposed by γ-lactams in peptides have been studied using valence force field energy calculations and flexible geometry maps. It has been found that while cyclisation restrains the ψ of the lactam, non-bonded interactions contribute to the constraints on φ of the lactam. The γ-lactam also affects the (φ,ψ) of the residue after it in a peptide sequence. For an L-lactam, the ring geometry restricts ψ to about −120°, and φ has two minima, the lowest energy around −140° and a higher minimum (5 kcal/mol higher) at 60°, making an L-γ-lactam more favourably accommodated in a near extended conformation than in position 2 of a type II′ β-turn. The energy of the $\varphi \approx +60°$ minimum can be lowered substantially until it is more favoured than the −140° minimum by progressive substitution of bulkier groups on the amide N of the L-γ-lactam. The (φ,ψ) maps of the residue succeeding a γ-lactam show subtle differences from those of standard N-methylated residues. The dependence of the constraints on the chirality of γ-lactams and N-substituted γ-lactams, in terms of the formation of secondary structures like β-turns is discussed and the comparison of the theoretical conformations with experimental results is highlighted.

INTRODUCTION

The incorporation of constraints into a peptide backbone has been widely used in an effort to increase activity and to better understand the structure–activity relationships of bio-active peptides [1,2]. The aim of this is to limit the conformational freedom of the peptide, so as to stabilise

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a desired conformation. This allows one to study whether the said conformation is in fact the elusive bio-active conformation. One of the ways of introducing constraints into a peptide backbone is by cyclising the side chain to the backbone as is the case in amino acid analogues involving lactams.

γ-Lactams have recently found increasing use in the design of peptides to impose constraints on the backbone [3–7]. Analogues of many important bio-active peptides like luteinising hormone releasing hormone (LHRH) [3], and Pro-Leu-Gly-NH_2 (which displays a range of pharmacological functions [8] in the central nervous system) incorporate γ-lactams [7]. The presence of the lactam ring was thought to restrict conformational freedom and force the peptide to adopt a β-turn [3]. Interactive computer graphics and energy minimisations on lactams of different ring sizes were used to support this claim [9]. However, the results reported by Freidinger [9] concentrated on the conformations of the rings alone and were not complete in terms of all torsional parameters responsible for the formation of β-turns. Recently the crystal structures of the peptide Pro-Leu-Gly-NH_2 and its γ-lactam modified analogues (where the γ-lactam forms a bridge between the Leu and Gly residues) have appeared in the literature [10,11]. The results show that, while Pro-Leu-Gly-NH_2 is characterised by a type II β-turn the γ-lactam modified peptides display extended or semi-extended conformations, except in one case where the lactam has an aminal imidazolidinone group and is in a type II' β-turn. Thus, the idea that γ-lactams favour β-turn formation in peptides is questionable.

Therefore, the conformational preferences of γ-lactams were studied using energy calculations. In particular, the feasibility of γ-lactams occurring in different secondary structures such as β-turns, and extended structures was explored. This has been achieved by calculating flexible geometry conformational energy maps of a γ-lactam, and of a Gly or an L-Ala residue following a γ-lactam.

METHOD

Energy calculations were performed using the valence force field [12, 13] in which the potential energy of a molecular system is represented as an empirical function of internal (valence) degrees of freedom and interatomic distances. The expression for the energy contains terms representing strain energies which arise from deformations of internal coordinates (like bond length, bond angle, and torsion angle) and 'cross terms' caused by coupling between deformations of two or more internals. The energy expression also has three terms representing the exchange repulsion, dispersion, and coulomb interactions between the nonbonded atoms. The details of the analytical form of the expression and the parameters used have been published elsewhere [14]. The geometry of the γ-lactam was taken from known crystal structure data [10]. Most of the energy parameters used in the calculations of the γ-lactam system were taken from a transferable set [14] except for the charges. The charges on the γ-lactam were obtained from those of related residues such as leucine and proline, and were modified slightly to keep the whole system neutral. The charges used, in fractions of the electron charge, were (with reference to Fig. 1) $N_1(-0.5)$, $H(N_1)(0.28)$, $C_1^{\alpha}(0.12)$, $C^{\beta}(-0.2)$, $C^{\gamma}(-0.2)$, $C'(0.38)$, $O(-0.38)$, $N_2(-0.5)$, $C_2^{\alpha}(0.2)$ and (0.1) on all other hydrogens.

Flexible geometry rotational barrier plots and (φ,ψ) energy maps were calculated by performing constrained minimisations at grid points every 10° or 20° (depending on the size of the system

and/or the number of variable torsions), in which all internal degrees of freedom were relaxed except for the angle of rotation of φ and ψ, which were constrained to adopt specific values by a harmonic forcing potential [15].

RESULTS AND DISCUSSION

The results of the geometrical arguments and the energy calculations on the γ-lactam systems are presented here starting from the parameters that affect the ring conformation. A schematic representation of a γ-lactam showing the different conformational parameters is given in Fig. 1. Since a γ-lactam is a bridge across two peptide units there are two nitrogens associated with it, one at the amino end and another in the ring. In this paper, we shall refer to N_1 (see Fig. 1) as the amide nitrogen of a γ-lactam residue and N_2 (which is the amide nitrogen of the residue following a γ-lactam) as the ring nitrogen. From the figure it can be seen that two backbone torsion angles, namely ψ and ω, are defined in the lactam ring. These torsions therefore show limited freedom of rotation. The values that these torsions can assume are discussed in detail below.

ω of a γ-lactam

It is fairly obvious that for cyclisation of the lactam-ring to be a possibility, the substituent on the ring nitrogen N_2 (see Fig. 1) of the γ-lactam (i.e. C^γ) must be *cis* to the C^α with respect to the C'-N_2 bond. This would mean that the peptide unit is in a *trans* conformation. Alternatively, from geometrical considerations it can be seen that the angle C_1^α-C'-N_2-C^γ will have to be close to 0°, as it is part of a 5-membered ring. This would make the angle C_1^α-C'-N_2-C_2^α, (i.e. ω), close to 180°. Thus the peptide bond in a γ-lactam is always in a *trans* conformation because of the constraint imposed by the cyclisation.

ψ of a γ-lactam

The argument for ψ (N_1-C_1^α-C'-N_2 in Fig. 1) follows a pattern similar to that outlined above for the ω of a γ-lactam. The C_1^α-C' bond about which ψ is defined is part of the ring and therefore any torsion about this bond will be restricted. In particular the ring torsion C^β-C_1^α-C'-N_2 is close to 0° as it is part of a 5-membered ring. This torsion is related to ψ by ± 120° because of the tetrahedral nature at C_1^α. The sign of this relative orientation is dependent on the chirality at C_1^α. In particular,

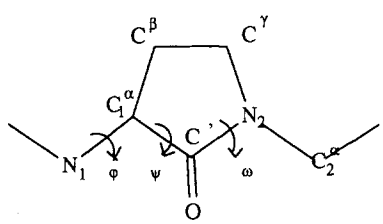

Fig. 1. Schematic representation of a γ-lactam with its conformational parameters defined.

the difference between the torsion angles C^{β}-C_1^{α}-C′-N_2 and N_1-C_1^{α}-C′-N_2 (ψ) is $+120°$ for an L-γ-lactam and $-120°$ for a D-γ-lactam. These arguments can be used to deduce the value of ψ for a γ-lactam and this is illustrated below for an L-γ-lactam. From Fig. 1, it can be seen that

C^{β}-C_1^{α}-C′-$N_2 \approx 0°$ – torsion angle in a 5-membered ring ..(1)

and N_1-C_1^{α}-C′-N_2 (i.e. ψ) $\approx$ C^{β}-C_1^{α}-C′-$N_2 - 120°$ – for an L-γ-lactam ..(2)

therefore $\psi \approx -120°$ – for an L-γ-lactam from (1) and (2)

Thus, the cyclic nature of the γ-lactam constrains the value of its ψ torsion angle. This is as shown above, around $-120°$ for an L-γ-lactam and (by symmetry) would be around $+120°$ for D-γ-lactam. Interestingly, a ψ of $-120°$ is unusual for a standard L-amino acid residue. Calculations [16–18] on a model system of an L-Ala dipeptide (N_1-acetyl-N_2-methyl-alanine) show that a ψ value between $-90°$ and $-160°$ is a high energy conformation. This is owing to the bad interactions between the C^{β} group and the NH group affected by the ψ rotation. In the special case of a γ-lactam however, such interactions are precluded as the substituent on the N, i.e. the C^{γ} (see Fig. 1) is bonded to the C^{β}.

φ of a γ-lactam

The conformational parameter φ being outside the ring (unlike ψ and ω) is not constrained geometrically and therefore full rotation about this torsion is possible. The conformational preferences of φ are expected to be guided largely by non-bonded interactions. Thus, the variation of the energy with the torsion φ, i.e. the φ rotational barrier for a γ-lactam was studied using flexible geometry calculations. Since crystal structure data for two types of γ-lactams are available [10, 11] in which the major difference lies in the substitution at the amide nitrogen (N_1 in Fig. 1), the effect of different substitutions at this N on φ was also studied. Four model systems were chosen to study the rotational barrier of φ. These four differ mainly in the substitutions at the nitrogen (N_1 of Fig. 1) defining the φ torsion angle. The models are

(a) N_1-acetyl-N_2-methyl-L-γ-lactam (3-acetamido-1-methylpyrrolidine)

(b) N_1-acetyl-N_2-methyl-(N_1-methyl)-L-γ-lactam (3-[(N-methyl)-acetamido]-1-methylpyrrolidine)

(c) N_1-acetyl-N_2-methyl-(N_1-*t*-butyl)-L-γ-lactam (3-[(N-*t*-butyl)-acetamido]-1-methylpyrrolidine) and

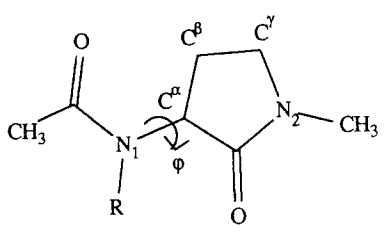

Fig. 2. Common schematic representation of γ-lactam systems (a–c), (a) R = H, N_1-acetyl-N_2-methyl-L-γ-lactam; (b) R = CH_3, N_1-acetyl-N_2-methyl-(N_1-methyl)-γ-lactam; (c) R = *t*-Bu, N_1-acetyl-N_2-methyl-(N_1-*t*-butyl)-γ-lactam.

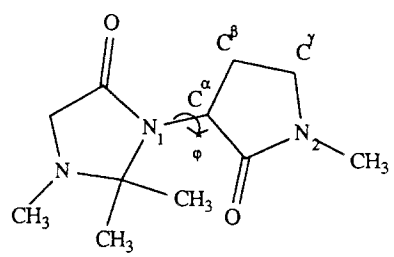

Fig. 3. Schematic representation of N_2-methyl-(N_1-imidazolidinone-γ-lactam.

TABLE 1
RELATIVE ENERGIES (in kcal/mol) OF THE TWO MINIMA AND THE ENERGY BARRIERS BETWEEN THEM IN THE γ-LACTAM MODEL SYSTEMS

Model systems	Energy minimum near φ of		Energy barrier
	$-140°$	$+60°$	
N_1-acetyl-N_2-methyl-L-γ-lactam	0.0	5.5	9
N_1-acetyl-N_2-methyl-(N_1-methyl)-L-γ-lactam	0.0	4.5	15
N_1-acetyl-N_2-methyl-(N_1-*t*-butyl)-L-γ-lactam	0.0	0.6	17
N_2-methyl-(N_1-imidazolidinone)-L-γ-lactam	0.25	0.0	13

(d) N_2-methyl-(N_1-imidazolidinone)-L-γ-lactam (1,2,3-trimethylimidazolin-4-one-(N-3)-(1-methylpyrrolidin-2-one-3-yl)

Two of the above four model systems, (a) and (d), are representative of the two types of γ-lactams for which crystal structure data is available. A common schematic representation of the first three model systems (a–c) above is given in Fig. 2, and the model system (d) is shown in Fig. 3.

Flexible geometry energy calculations were performed on these four model systems and the rotational barrier of φ was monitored. This was done by varying the value of φ within the range

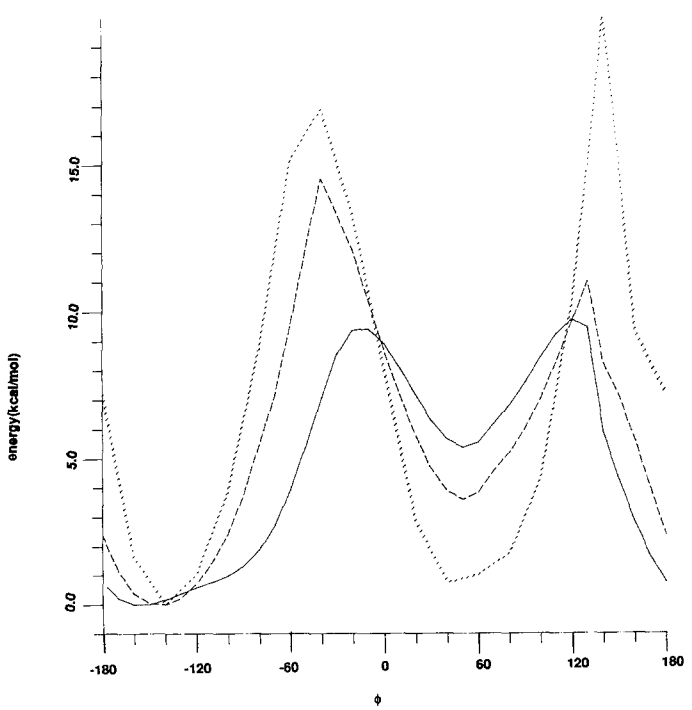

Fig. 4. The φ-rotational barrier of N_1-acetyl-N_2-methyl-L-γ-lactam (solid line), N_1-acetyl-N_2-methyl-(N_1-methyl)-γ-lactam (dashed line), and N_1-acetyl-N_2-methyl-(N_1-*t*-butyl)-γ-lactam (dotted line).

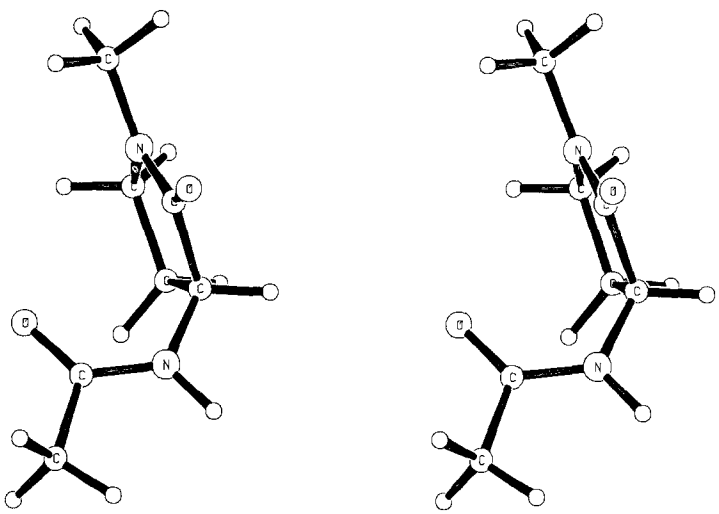

Fig. 5. Stereo plots of N_1-acetyl-N_2-methyl-L-γ-lactam in a minimised conformation with $\varphi \approx +60°$ showing the interaction of the carbonyl group with the lactam ring.

$-180°$ to $180°$ in $10°$ increments. The starting geometry for the γ-lactam was taken from crystal structure data [10], in which the value of ψ was $-130°$. Since ψ is constrained by the lactam ring, it was not necessary to use a torsion-forcing potential to fix it to any particular value.

The results of the fully flexible minimisations are summarised in Table 1. The results show that, in all the four cases, two minima exist for φ values of $\approx -140°$ and $\approx +60°$. The relative energies of the two minima in each of the four cases and the barrier between them are given in the table. The model systems as represented by Fig. 2 are discussed first. Fig. 4 shows the φ rotational barrier for N_1-acetyl-N_2-methyl-L-lactam, N_1-acetyl-N_2-methyl-(N_1-methyl)-L-lactam, and N_1-acet-

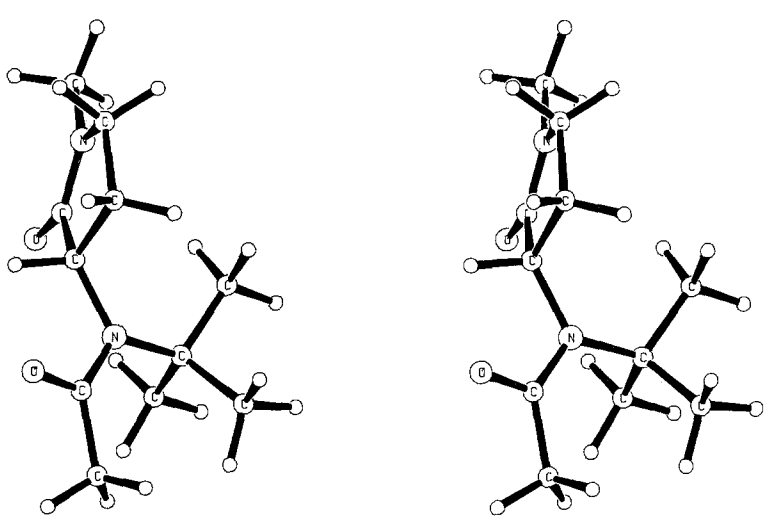

Fig. 6. Stereo plots of N_1-acetyl-N_2-methyl-(N_1-*t*-Bu)-L-γ-lactam in a minimised conformation with $\varphi \approx -140°$ showing the interaction of the amide nitrogen substituent with the lactam ring.

yl-N_2-methyl-(N_1-*t*-butyl)-L-lactam. These are represented as solid, dashed, and dotted lines, respectively. In the case of N_1-acetyl-N_2-methyl-L-lactam (solid line in Fig. 4), the minimum at $\varphi \approx -140°$ is at least 5 kcal/mol more stable than the one at $+60°$. As the size of the N_1-substituent increases, the difference in energy between the two minima decreases as in the case of N_1-acetyl-N_2-methyl-(N_1-methyl)-L-lactam (dashed line), and indeed for the very bulky substituent viz. N_1-acetyl-N_2-methyl-(N_1-*t*-butyl)-L-lactam (dotted line) the minimum at $+60°$ is nearly as low as the $\varphi \approx -140°$ minimum. Furthermore, the barrier to rotation increases as the size of the substituent increases and the two minima become more sharply defined.

In terms of the underlying interactions that affect the two minima of φ in the three systems discussed above, interactive computer graphics shows us that the φ-dependent destabilising interactions (bearing in mind that the ψ is fixed to around $-120°$) are one of the two described below.

(1) In the $\varphi \approx +60°$ conformation, the predominant high-energy interactions are between the carbonyl of the acetyl group and the lactam ring. This is shown in Fig. 5.

(2) In the $\varphi \approx -140°$ conformation, the predominant high-energy interactions are between the substituent at the amide N (N_1 in Fig. 1) and the lactam ring as seen in Fig. 6.

Thus the competition between the two sets of interactions determines which of the two φ minima is preferred in each of the model systems studied. As the substituent on the N_1 gets bulkier a second set of interactions becomes important, i.e. those involving the lactam ring with the substituent on N, which destabilise the $\varphi \approx -140°$ minimum relative to the $+60°$ minimum.

The case of N_2-methyl-(N_1-imidazolidinone)-L-lactam as represented in Fig. 3 is discussed next. This model system has been specifically included in the calculations as a crystal structure of a

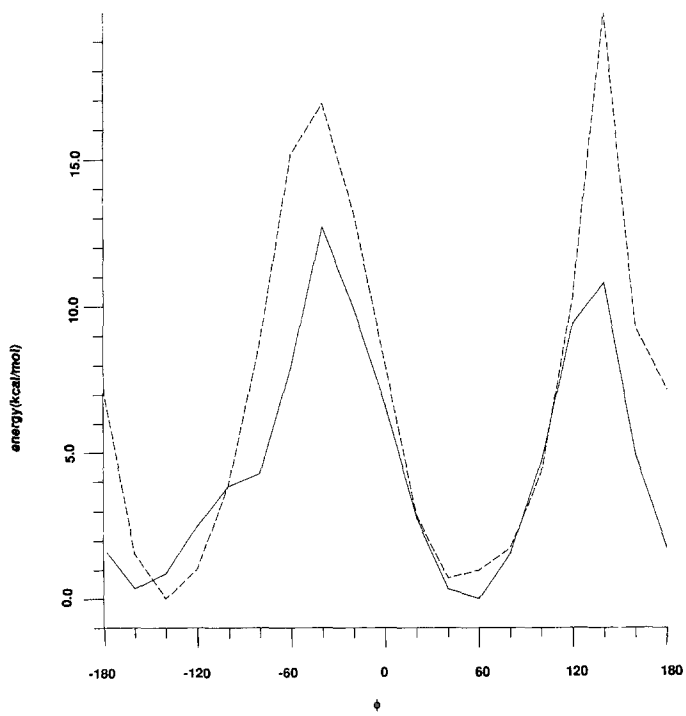

Fig. 7. The φ-rotational barriers of N_2-methyl-(N_1-imidazolidinone)-γ-lactam (solid line), and N_1-acetyl-N_2-methyl-(N_1-*t*-butyl)-γ-lactam (dashed line).

closely related system is available [10]. The variation of energy with φ for this model system shows subtle differences from the other φ rotational barriers discussed above. The variation of energy with φ here is shown as a solid line in Fig. 7. For comparison this figure also gives the φ rotational barrier of N_1-acetyl-N_2-methyl-(N_1-*t*-butyl)-L-lactam (dashed line). From the two φ rotational barriers in Fig. 7 it can be seen that, though the two systems have just as bulky amide substitutions, the N_2-methyl-(N_1-imidazolidinone)-L-lactam (solid line) differs in its conformational features slightly from the N-*t*-butyl model system (dashed line). Specifically, the $\varphi \approx +60°$ minimum is lower in energy than the $-140°$ minimum and the barrier to rotation considerably lower. The sources of these differences have been studied using interactive computer graphics. It is found that the cyclisation requirements at the imidazolidinone forces the torsion about the N_1-C(*t*-butyl) bond to adopt fixed values. Fig. 8 shows the N_2-methyl-(N_1-imidazolidinone)-L-lactam in a minimised conformation with $\varphi \approx -140°$. A comparison between Figs. 8 and 6 shows that the interactions involving the carbonyl of the lactam ring with the methyl groups on the butyl carbon are more destabilising for the imidazolidinone model than for the corresponding conformation in the N-*t*-butyl case.

So far we have concentrated on the parameters that affect the conformation of the γ-lactam *per se*. However, γ-lactams are bridges across two amino acid residues and are therefore expected to affect the conformation of the next residue in the sequence.

Conformational constraints on the residue succeeding a γ-lactam

The constraints imposed by a γ-lactam on an amino acid following it were studied using flexible geometry techniques by calculating the (φ,ψ) maps of Gly and Ala. Since the Gly or Ala succeeding a γ-lactam is similar but not identical to an N-methylated Gly dipeptide, the effects of N-methylation and of cyclisation were examined separately. In the first instance the flexible geometry (φ,ψ) maps of an N-methylated Gly and an N-methylated Ala were computed. A schematic repre-

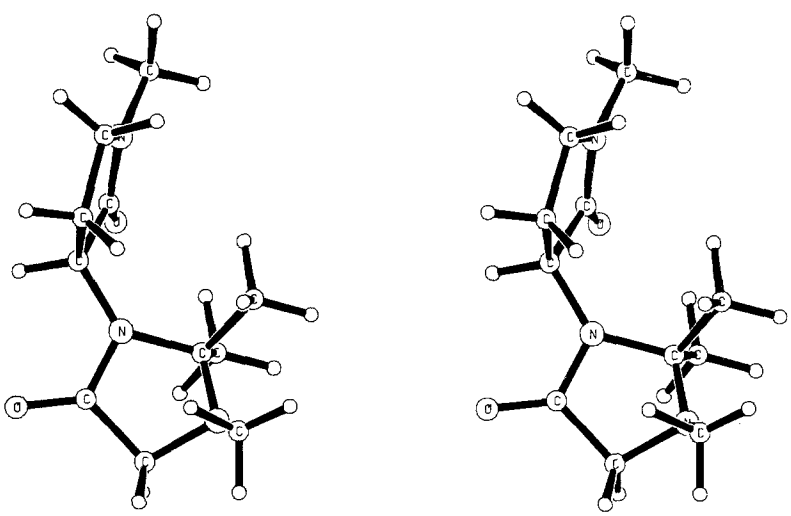

Fig. 8. Stereo plots of N_2-methyl-(N_1-imidazolidinone)-γ-lactam in a minimised conformation with $\varphi \approx -140°$ showing the interaction of the imidazolidinone group with the lactam ring.

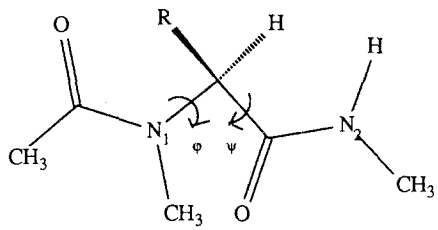

Fig. 9. A schematic representation of an N_1-methylated dipeptide, R = H for Gly; R = CH_3 for Ala.

sentation of the N-methylated systems is shown in Fig. 9 and the (φ,ψ) maps for Gly and Ala are shown in Figs. 10A and B respectively. These maps are slightly different from the well known standard Gly and Ala maps. The major difference between these maps (Figs. 10A and B) and their standard counterparts [16–18] is that the extended region becomes a relatively high energy conformation and the α-helical region is considerably narrowed. There are, as can be seen from Figs. 10A and B, two narrow bands of allowed conformations centered around $\varphi \pm 100°$.

In order to study the additional constraints imposed by the lactam ring on an amino acid residue following it (see Fig. 11) we have also computed the flexible geometry (φ,ψ) maps of Gly and Ala using the model systems L-lactam-Gly and L-lactam-Ala. These are shown in Figs. 12A and B, respectively. These figures show subtle differences from the N-methylated Gly and Ala maps (Figs. 10A and B) and the differences are discussed below.

A comparison of the (φ,ψ) maps of an alanine following a γ-lactam (Fig. 12B), and an N-methyl Ala dipeptide (Fig. 10B) indicates that, for the L-lactam-Ala system

(1) the extended region is less favoured as exemplified by the 6 kcal/mol contour which encloses the low-energy regions.

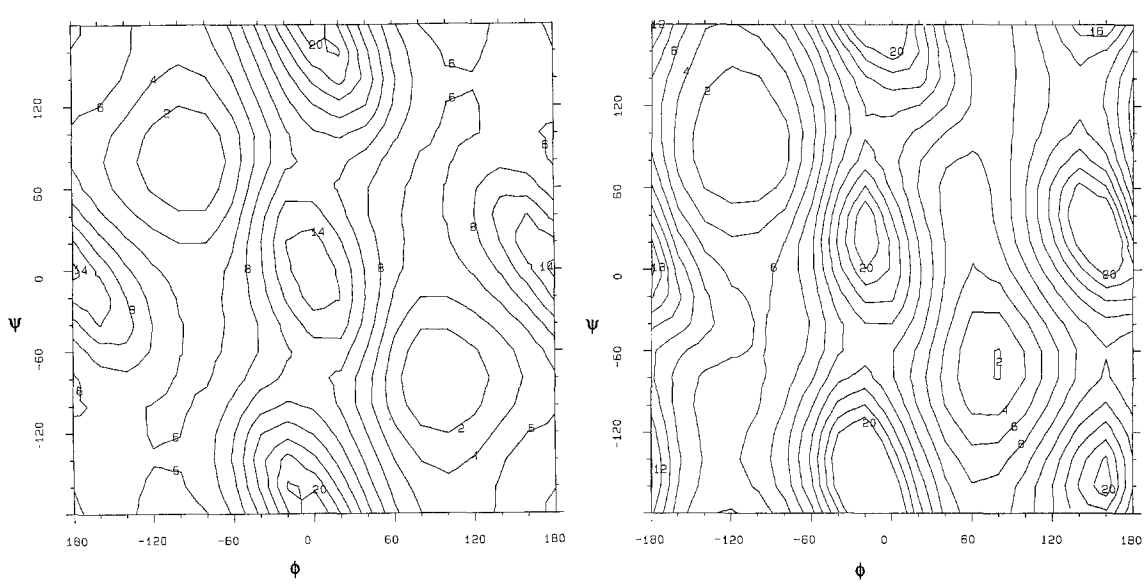

Fig. 10. (φ,ψ) maps of (A) N_1-methylated Gly dipeptide and (B) N_1-methylated L-Ala dipeptide. Relative energy contours are drawn at 2 kcal/mol intervals.

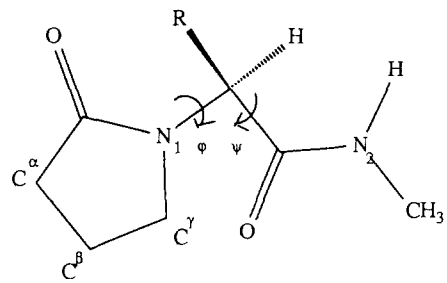

Fig. 11. Schematic representation of model systems. R = H, L-γ-lactam-Gly-N_1-methyl; R = CH_3, L-γ-lactam-L-Ala-N_1-methyl.

(2) the low-energy regions have been pushed toward $\psi = 0°$.

(3) the region around the α-helix, i.e. $(\varphi,\psi) \approx (-50°, -50°)$, has broadened and become more allowed.

Similar differences were found in the Gly case (Figs. 10A and 12A). However, the effect here is not as pronounced as in the L-lactam-Ala dipeptide. Clearly, the presence of a bulky substituent like a methyl group on the amide nitrogen of Gly and Ala makes most of the semi-extended and extended regions high in energy. The exclusion of these conformations is mainly due to the high-energy interactions between this methyl on the amide N (see Figs. 9A and B) and the carbonyl group. However, most of the differences in the (φ,ψ) maps of Gly and Ala systems following a γ-lactam are a result of the lactam ring which does not allow rotation about the N_1-C^γ bond (see Fig. 11).

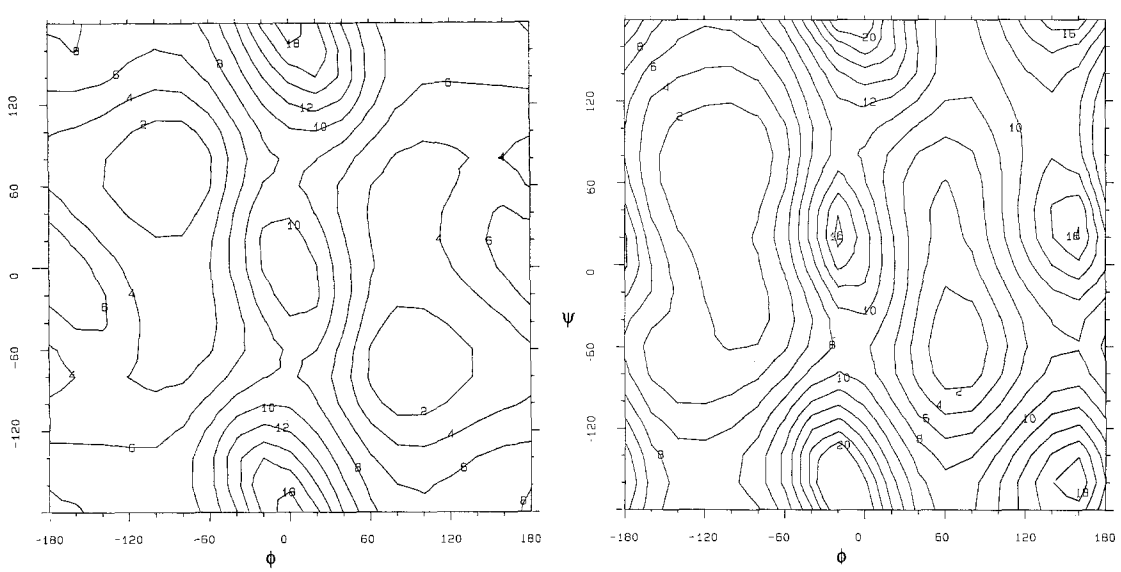

Fig. 12. (φ,ψ) maps of (A) N_1-L-γ-lactam-Gly-N_1-methyl and (B) N_1-L-γ-lactam-Ala-N_1-methyl. Relative energy contours are drawn at 2 kcal/mol intervals.

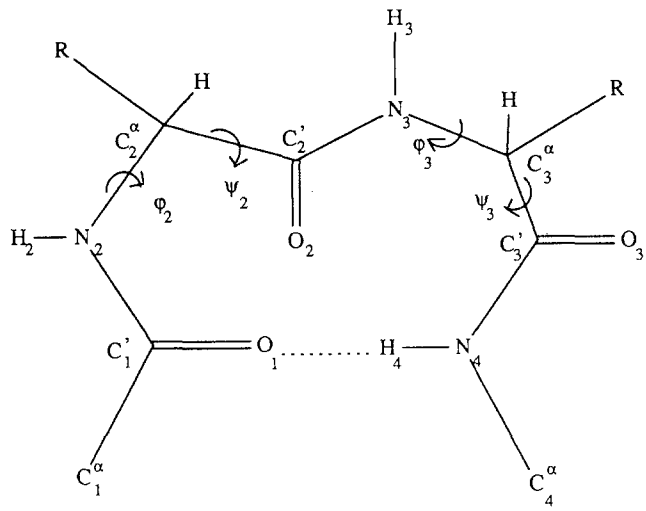

Fig. 13. A schematic representation of a β-turn showing all relevant conformational parameters. The 4→1 hydrogen bond is shown by a dashed line.

γ-lactams and β-turns

β-Turns [19] are well known secondary structures in peptides and proteins [20] which cause a reversal of a peptide chain over a sequence of four residues. These are stable structures and are often associated with a hydrogen bond between residues 4 and 1. Conformationally a β-turn is fully defined by the (φ,ψ) torsions of the middle two residues viz. (φ_2,ψ_2) and (φ_3,ψ_3). Fig. 13 shows a schematic representation of a β-turn with the conformational parameters defined. The formal definitions of types I–III β-turns and their mirror images are given in Table 2. Here, the accommodation of γ-lactams in β-turns is discussed.

To start with, the ψ of the γ-lactam is one parameter which can be predicted with confidence from geometrical constraints and is not expected to show a large variation as it is within a ring. For an L-γ-lactam this value is $-120°$ (here the arguments are presented for an L-γ-lactam which

TABLE 2
CHARACTERISTIC TORSIONS OF TYPES I–III AND I′–III′ β-TURNS [19]

β-Turn type	φ,ψ values in (°) at position 2 and 3			
	φ_2	ψ_2	φ_3	ψ_3
I	−60	−30	−90	0
II	−60	+120	+80	0
III	−60	−30	−60	−30
I′	+60	+30	+90	0
II′	+60	−120	−80	0
III′	+60	+30	+60	+30

can from symmetry be extended to a D-γ-lactam). By inspection of the ψ_2 and ψ_3 values given in Table 2, it can be seen that position 3 can be completely ruled out in all the β-turns as a value close to 0° is required, and the only position that an L-γ-lactam could occupy is position 2 in a type II'β-turn. The deduced value of ψ of the γ-lactam matches exactly the required ψ_2 of this turn.

Having shown that the ψ of an L-γ-lactam is compatible with position 2 of the type II' β-turn, attention is focused on its φ value. The required φ_2 value of a type II' β-turn is $+60°$. The L-γ-lactam has two minimum energy values for φ. These are $-140°$ and $+60°$, as can be seen in Table 1. Although the $+60°$ conformation is compatible with the requirement for φ_2 in a type II' β-turn, this is the higher energy conformation. As seen before (Fig. 4) the minimum around $\varphi \approx -140°$ is at least 5 kcal/mol lower in energy than the $+60°$ conformation for an L-γ-lactam.

As regards the conformation of a residue succeeding the lactam in the turn, i.e. φ_3 and ψ_3, it can be seen from Table 2 that φ_3 and ψ_3 should have values near $-80°$ and 0° respectively for a type II' β-turn. From the (φ,ψ) flexible geometry maps of both Gly and Ala following an L-γ-lactam (these are given in Figs. 12A and B), we find that these values are particularly favoured because of the clustering of low-energy conformations around the $\psi = 0°$ line. Therefore, while most of the parameters of the pair of residues are compatible with a type II' β-turn, the φ of the L-γ-lactam is not, in that the $\varphi \approx -140°$ minimum is lower in energy than the $+60°$ minimum. Thus, for a γ-lactam-Xaa system (where Xaa is Gly or Ala) a β-turn is not the most favoured conformation.

However, the $\varphi \approx +60°$ minimum can be made more favourable by the substitution of bulky groups on the amide nitrogen of the lactam. By such an appropriate substitution it is possible for the L-γ-lactam to reproduce the required (φ_2,ψ_2) values of a type II'-β-turn. Thus to form a β-turn (specifically a type II' β-turn), an N-substituted L-γ-lactam should be used, and for a type II β-turn an N-substituted D-γ-lactam is required.

It is worthwhile to note that a γ-lactam is not suitable in position 4 of a β-turn. This is owing to the bad clashes introduced by the substituent on the N_4 (see Fig. 13) viz. C^γ of a γ-lactam, with the carbonyl of the residue in position 1 of the turn.

Dependence of β-turn type on γ-lactam chirality

In the previous section it was shown that for a γ-lactam to be accommodated in β-turns it has to be suitably modified. It was also shown that a modified L-γ-lactam could form only a type II' β-turn and a modified D-γ-lactam a type II β-turn. This effect of the chirality of the γ-lactam on the preference of β-turn type is discussed here. It is assumed that the γ-lactam is appropriately modified (i.e. substitutions of bulky groups on the amide nitrogen) such that the $\varphi \approx \pm 60°$ minimum is preferred and that the β-turns can be formed. The arguments are mainly presented for an L-γ-lactam and these can be extended to a D-γ-lactam by symmetry.

For a modified L-γ-lactam, the β-turn forming conformation is $(+60°, -120°)$ at position 2 of the turn. This conformation is unusual for a standard L-amino acid residue (taking 'standard' to mean an amino acid that occurs in proteins), though it is a low-energy conformation for a standard D-amino acid residue. Similarly, for the D-γ-lactam the β-turn forming conformation having a (φ,ψ) of $(-60°, 120°)$, is more commonly associated with a standard L-residue. This predilection of an L-γ-lactam for a conformation which is low energy for a standard D-residue and *vice versa*, means that it departs from the usual chirality-sequence β-turn preference rules [21]. In particular, for a type II' β-turn, a standard D-amino acid in position 2 is preferable, whereas with a modified

γ-lactam an L-form is preferred according to the results presented in this paper. This is, as we have shown, due to certain high-energy interactions being precluded as a consequence of the lactam ring. The dependence of the chirality of γ-lactams to β-turn type as highlighted here is validated by the crystal structure data [10, 11] on γ-lactam containing peptides.

Comparison with experimental results

X-ray data is available on a few peptides incorporating γ-lactams [10, 11]. Here a comparison is made between the crystal structure data and the conformations predicted from our calculations. The (φ,ψ) values of the γ-lactam from crystal structure data are given in Table 3 alongside theoretical values from our calculations. The chirality of the lactam and the peptides in which they occur are also given. It can be seen from the table that the (φ,ψ) values of the γ-lactams in the crystal structure are consistent with the calculations reported here. For instance, the value of ψ of the γ-lactams is close to $\pm 120°$. This is as has been predicted from geometrical considerations close to $-120°$ for an L-γ-lactam and $+120°$ for the D-γ-lactam. Now considering the φ of the γ-lactam, the crystal structures have values near the calculated preferred minimum of $-140°$ for the L-γ-lactam ($\varphi = -115.7°$), $+140°$ for the D-γ-lactam ($\varphi = 117.9°$), and near $+60°$ ($\varphi = 57.4°$) for a modified L-γ-lactam (it must be noted here that for the modified L-γ-lactam, i.e. with an imidazolidinone group, the conformation $(-140°, -120°)$ is only marginally higher in energy than the $(+60°, -120°)$ minimum). Thus the agreement between the experimental conformational values of a γ-lactam and those obtained from the calculations reported here is excellent.

In the crystal structures of all the three peptides containing γ-lactams, the residue following the γ-lactam is Gly which is also the C-terminal end of the peptides. The (φ,ψ) values of Gly are found to be $(78.7°, -144.4°)$, $(-92.3°, 6.8°)$ and $(119.2°, 150.9°)$. At first glance only the conformation $(-92.3°, 6.8°)$ seems to be consistent with the calculated (φ,ψ) map (Fig. 12A) while the other two are in relatively higher energy conformations. In particular the predicted value of ψ close to 0° is not found in these two cases. However, closer inspection of the crystal structures of these two

TABLE 3
COMPARISON OF CRYSTAL STRUCTURES [10, 11] OF γ-LACTAMS WITH THEORETICAL CONFORMATIONS

Peptide	γ-Lac chirality	Conformation of γ-lactam			
		Crystal structure (exact)		Theoretical conformation (approximate)	
		$\varphi°$	$\psi°$	$\varphi°$	$\psi°$
Pro-γ-Lac-Gly-NH_2	D	117.9	141.9	140	120
Pro-γ-Lac-Gly-NH_2 (modified γ-lactam[b])	L	57.4	−129.9	60	−120[a]
Boc-Pro-γ-Lac-Gly-NH_2	L	−115.7	−131.7	−140	−120

[a] It must be noted that the $(-140°, -120°)$ minimum is only marginally higher in energy (refer Table 1).
[b] Modified by imidazolidinone group at the γ-lactam.

peptides shows that the crystal packing requirements [10, 11] have forced the NH_2 following the Gly residue to adopt such conformations. Indeed, in the crystal structures all the NHs, following Gly participate in inter-molecular hydrogen bonds. Also, the calculations here are more relevant to Gly succeeding a γ-lactam where the peptide chain extends beyond the Gly (see Fig. 11) than to the case as represented by the crystal structures where Gly is the C-terminal end.

In terms of overall structure, two of the peptide systems containing γ-lactams show semi-extended conformations, while the peptide containing a modified L-γ-lactam forms a type II' β-turn conformation. All these peptides containing γ-lactam are modifications of a biologically active parent peptide Pro-Leu-Gly-NH_2, which is found in the crystal form in a type II β-turn [11, 22] conformation. This difference in conformation between the parent peptide and its γ-lactam-modified analogues, in terms of (a) presence of β-turn, (b) type of turn when present, and (c) influence of chirality of residues affecting β-turn type, is easily explained by the different sections in this paper. This is demonstrated below for the peptide Pro-Leu-Gly-NH_2.

Pro-Leu-Gly-NH_2 is a bio-active peptide involved in a host of pharmacological functions [8]. It exists in a type II Leu-Gly β-turn conformation as evidenced by the crystal structure [11, 22] data. Pharmacological experiments show that the analogue Pro-D-γ-Lac-Gly-NH_2 is 10 000 times more active than the native peptide, in enhancing the binding of a dopamine agonist [7] while the L-γ-Lac analogue is inactive. This led to the structural implication that the D-γ-Lac analogue would have a conformation similar to that of the parent peptide and would constrain the peptide into a type II β-turn. To the extent that the ψ of a γ-Lac required for a type II β-turn is around $+120°$ (see Table 2), the chirality of the γ-lactam is appropriate, namely only a D-γ-lactam can have such a ψ value. But for the D-γ-lactam to be in the position 2 of a type II β-turn, it must have a φ value around $-60°$ (Table 2). However, according to our calculations, since the D-γ-lactam is not modified at its amide nitrogen, it will prefer a φ value of $+140°$ over a φ value of $-60°$ making a near extended conformation more favourable than the position 2 of a type II β-turn. (The arguments for the D-γ-lactam here have been derived from the conformational preferences of an L-γ-lactam by symmetry.) This is validated by experimental data. The crystal structure of Pro-D-γ-Lac-Gly-NH_2 shows that the peptide exists in a near extended conformation [10].

The possible conformations of a luteinising hormone-releasing hormone (LHRH) antagonist have recently been worked out from molecular dynamics simulations [23]. The structural implication of γ-lactams in LHRH, specifically in the context of β-turn formation, is discussed in that paper.

CONCLUSION

In conclusion, incorporation of an L-γ-lactam into a peptide sequence will result in the following.

First, it will introduce a value of $\psi = -120°$ into the peptide backbone. It will also prefer a φ value of $-140°$, but, a φ of $+60°$ can be forced by the inclusion of bulky groups (for instance *t*-butyl) on the amide nitrogen defining this torsion. As a consequence of this it can be seen that β-turn conformations are not favourable for a γ-lactam unless appropriately modified. If such a modified L-γ-lactam is to promote a β-turn, then the only turn it could form is a type II' (conversely a modified D-γ-lactam can only form a type II β-turn), with the γ-lactam in position 2 of the four residues forming the turn.

Second, the lactam will affect the (φ,ψ) of the residue following it, making extended conformations disallowed, moving low-energy regions towards $\psi = 0°$, and also broadening the allowed α-helical region, thus making that residue suitable for position 3 of a β-turn. Furthermore, the effect of the lactam ring and the special conformational properties that it induces, makes peptides in β-turn conformations containing modified γ-lactams depart from accepted chirality-sequence β-turn preferences.

The results reported here are in excellent agreement with crystal structure data on γ-lactam containing peptides. It is hoped that this insight into the conformational preferences of γ-lactams in constraining peptide structure will be a useful tool in the rational design of bio-active peptides.

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