Design Requirements for Pyroglutamic Acid Substitutions in Peptides

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Conformational energy calculations and geometrical arguments indicate that L-pyroglutamic acid (Glp) is an unusual amino acid residue in that its backbone conformational requirements are similar to those normally associated with a standard D-amino acid residue.

The two commonly occurring cyclic amino acid residues in peptide hormones are Pro and Glp. In particular, Glp is found as the *N*-terminal residue in luteinising hormone releasing hormone (LHRH), thyrotropin releasing hormone (TRH) and in gastrointestinal hormones like gastrin, cerulein, neurotensin and bombesin. In both Pro and Glp, the presence of the cyclic side chain constrains the local conformation of the residues. However, the effect that these residues have on conformation (*e.g.* φ,ψ values) is remarkably different. It is well known, for example, that L-Pro restricts the φ value to near $-60°$. The effect of the ring structure in Glp is explored here and it is shown that it has backbone conformational characteristics similar to those normally associated with standard D-residues.

From geometrical arguments, a qualitative assessment can be made of the conformational parameters of a Glp residue. Considering Fig. 1, it can be seen that in a Glp residue the angle C^{δ}–N–C^{α}–C^{β} is $\pm 20°$ as it is part of a five-membered ring. Additionally, with respect to the N–C^{α} bond, any torsion angle involving C^{β} will be oriented $-120°$ relative to a torsion angle involving C′ for an L-amino acid residue with tetrahedral geometry at the C^{α} atom. Therefore the value of C^{δ}–N–C^{α}–C′ is expected to be close to $+100°$ or $+140°$. These arguments are summarised in Table 1. Indeed, a rigorous flexible geometry-minimisation of the C^{δ}–N–C^{α}–C′ angle done using the Valence Force Field [1] (VFF) shows (Fig. 2) two minima close to values of 110° and 140°. The difference between these two minima is

Table 1. Summary of arguments showing the conformational preference of an L-Glp

Torsion angle	Reason for value of angle
C^{δ}–N–C^{α}–C^{β} *ca.* $\pm 20°$	Torsion angle in a 5-membered ring (1)
C^{δ}–N–C^{α}–C^{β} = C^{δ}–N–C^{α}–C′ − 120°	For an L-Glp residue With tetrahedral geometry at C^{α} (2)
C^{δ}–N–Cα–C′ *ca.* $+100°$ or $+140°$	From (1) and (2) above

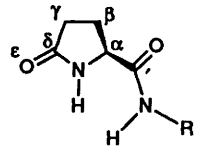

Fig. 1 Schematic representation of a pyroglutamic acid (Glp) residue. Note that the C^{β} atom can be above or below the plane of the paper depending on the angle C^{δ}–N–C^{α}–C^{β} being around +20° or −20°

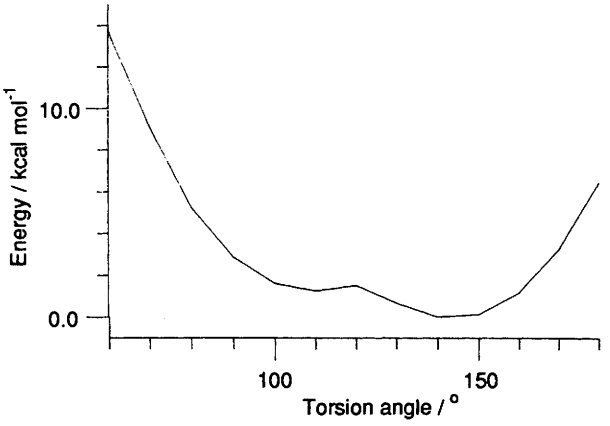

Fig. 2 Variation of energy with C^{δ}–N–C^{α}–C′ parameter (see Fig. 1) from flexible geometry energy calculations

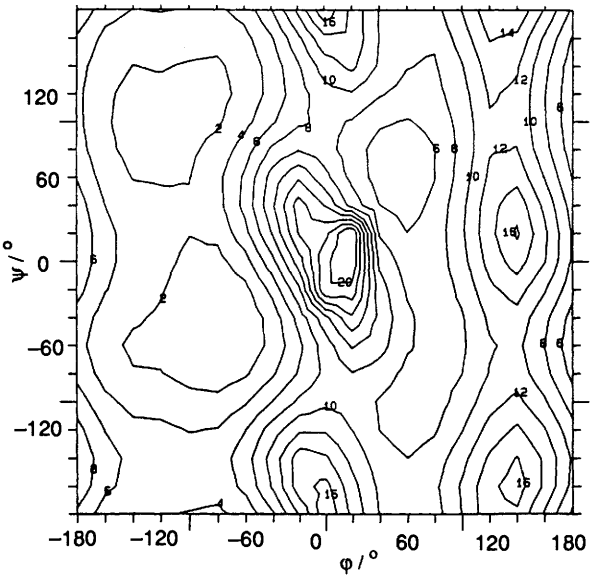

Fig. 3 Flexible geometry (φ,ψ) energy contour map of an *N*-acetyl-L-Ala-*N*-methylamide with the first peptide bond in *cis* and the second peptide bond in *trans* conformations

due to the conformational variety exhibited by the five-membered ring, in particular, in the disposition of the C^{β} group relative to the plane formed by the C^{γ}, C^{δ}, N and C^{α} atoms. This part of the ring is planar *i.e.* the torsion about the amide bond is 0° in the 110° and the 140° minima. This results in the other endocyclic torsion angles being the inverse of each other in the two cases. Conformationally, the difference shows up as the C^{β} group and the exocyclic carbonyl group being on opposite sides of the C^{γ}–C^{δ}–N–C^{α} plane in the 110° minimum and on the same side in the 140° minimum. A survey of crystal structures containing L-Glp (taken from the Cambridge Crystallographic database) shows values of C^{δ}–N–C^{α}–C′ to be mainly close to 140° with a few examples showing values of 100°. This angle,

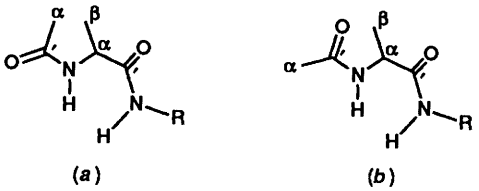

Fig. 4 Schematic representation of an *N*-acetyl-L-Ala with the first peptide unit in (*a*) *cis* and (*b*) *trans* conformation

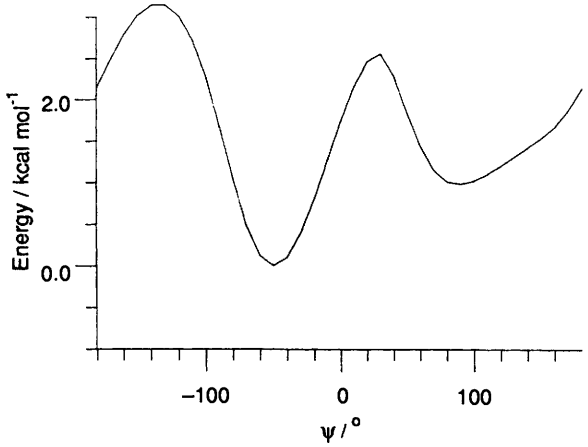

Fig. 5 Variation of energy with ψ of an L-Glp residue from flexible geometry energy calculations

however, would correspond to the definition of the φ torsion angle in a normal residue. This can easily be visualised by disregarding the C^{γ}–C^{β} bond in Fig. 1 which would immediately make this part resemble a peptide unit, albeit in the *cis* form, with the C^{γ} and C^{δ} atoms corresponding to the C^{α} and C′ respectively of the peptide unit. Alternatively, without the C^{γ}–C^{β} bond a Glp residue resembles an *N*-acetyl-Ala residue. It is well known from contact criteria and energy calculations on *N*-acetyl-L-alanine-*N*-methylamide[2–4] that a φ value between 100° and 140° is not a low energy conformation for an L-amino acid residue. This is shown here to be true even for an *N*-acetyl-L-alanine-*N*-methylamide having peptide bonds in the *cis* and *trans* forms respectively, as in the case represented by Glp. A flexible geometry (φ,ψ) map for an *N*-acetyl-L-alanine-*N*-methylamide with the two peptides in *cis* and *trans* conformations was calculated using the VFF[1] and this is shown in Fig. 3. Clearly even in this case values of φ in the range between 100° to 140° are quite high in energy and are at least 8–10 kcal mol^{-1} above the minimum.* The reason for this high energy is explained by Figs. 4(*a*) and (*b*) which represent the standard peptide unit (obtained by removing the C^{γ}–C^{β} bond of Glp in Fig. 1 and reassigning the C atoms) in the *cis* and *trans* forms. In a standard residue most of the short contacts are between the C^{α} (of the previous residue, corresponding to C^{γ} of Fig. 1) and C^{β} atoms and their associated hydrogens in the *cis* form and similarly between the O (of the previous residue) and C^{β} in the *trans* form. In the special case of Glp, however, these short contacts are non-existent as the C^{γ} is bonded to the C^{β} atom. Thus, for a φ value between +100° to +140°, unfavourable high energy interactions that would have been found between atoms of a standard L-residue are precluded in a Glp residue.

The variation of the torsion angle ψ of a Glp residue (angle N–C^{α}–C′–N in Fig. 1) has also been calculated and this is shown in Fig. 5. Two low energy regions of ψ can be identified; the lowest at around −40° and a higher shallow minimum around

* 1 cal = 4.184 J.

+100°. No discernible correlation between the two possible ring conformations and these two ψ values could be found. The conformational parameters of a Glp residue as worked out here are consistent with recent crystal structure analysis on peptides containing Glp.[5] Both these regions of conformational space (*i.e.* φ value of 100° or 140° with ψ of −40° or +100°) are high energy for a standard L-residue, but acceptable conformations for a standard D-residue. The immediate implication is that an L-Glp residue is conformationally equivalent to a standard D-amino acid residue in terms of its backbone conformational requirements.

Recently, we worked out the conformational constraints imposed by bridged γ-lactams which showed a good agreement with crystal structure studies.[6] It was shown that for a L-γ-lactam, the ring geometry restricts its ψ value to −120° and owing to certain nonbonded interactions φ has two minima; a shallow one near the extended conformation and a higher minimum near +60°. In terms of the (φ,ψ) values, the two regions of conformational space available for a bridged L-γ-lactam are stereochemically more conducive for a standard D-residue than for a L-residue. A Glp residue is a γ-lactam at the *N*-terminal end of a peptide, and the constraint imposed by the ring structure shows a similar effect on its backbone conformational preferences. However the preferences are for quite different reasons. In the bridged γ-lactam it is the ψ parameter that is constrained whereas in the Glp residue it is the rotational restriction about the N–C^{α} bond that is responsible for the peculiar characteristics.

Thus an L-Glp residue which resembles an *N*-acetyl-L-Ala residue with the acetyl group bonded to the Ala sidechain, has a conformation which would be high in energy for an standard L-residue but acceptable for a D-residue. Therefore, in the design of potent analogues of bioactive peptides, if the relative orientations of the backbone atoms or groups have an important bearing in terms of conferring increased binding affinity at the receptor site, any substitution of Glp by a standard amino acid residue should, we believe, be necessarily of the D-isomeric form. In particular, any substitution that would specifically constrain the conformation to (φ,ψ) values in the region of (+100°, −40° or +100°) and (+140°, −40° or +100°) and the peptide unit to a *cis* form would be an obvious choice for replacement. Studies relating to likely ring structures that would be ideal substitutions of a Glp residue, and the effect of such substitutions on the rest of a peptide in terms of the stability of formation of secondary structures like α-helices and β-sheets, are currently underway.

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