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REDUCED AND RETRO-REDUCED PEPTIDE ANALOGUES-CONFORMATIONS AND ENERGIES

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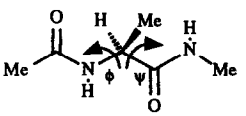
Summary: *A complete search of the conformational space available to residues with modified amide links was carried out by flexible geometry minimisations of a grid of values of all rotatable bonds. The implications for incorporating these residues in secondary structures were studied.*

Modified amino acid residues are of biological interest as "building blocks" in peptide chemistry. For example, hormone analogues-designing molecules with topochemical similarity to peptide hormones, such that receptor binding and biological response are retained, but with enhanced resistance to biodegredation; protease inhibitors - designing molecules that mimic the substrate or the substrate's hydrolysis transition state, but with a non-scissile bond; peptide engineering - inducing structural changes in peptides by selective substitution of residues by analogues with modified conformational preferences. Retro-inverso amide links have been incorporated successfully in bioactive peptides,[1] and reduced amide groups were used as transition state analogue inhibitors of renin.[2] As part of our combined experimental[3] and theoretical study of novel surrogates for the amide bond we present here the conformational consequences of introducing a reduced or retroreduced amide link in peptides.



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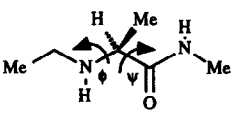

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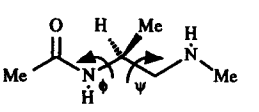

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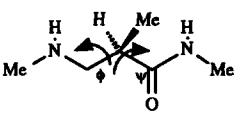

IV

V

Figure 1

The strategy used was to carry out a complete search of the conformational space available to the modified residues and then to investigate the implications for incorporating these residues in secondary structures. We used a blocked alanine residue (Fig. 1.I) as a model for the effect of reducing the carbonyl group and of reversing the amide bond direction on the conformation and energy of the residue. Since the modifications are at the bond bridging two residues, two analogues were studied for each modification, in order to elucidate the implications for the residue before and after the amide bond. The two model analogues for the reduced peptide are given in Fig 1.II and 1.III and the two model retroreduced peptides are given in Fig 1.IV and 1.V.[4]

The potential energy of the compounds studied was represented by a valence force field, with

parameters which were derived for peptides and proteins by fitting experimental data for small model compounds.[5] Flexible geometry ϕ,ψ energy maps were calculated using a full VFF expression and adding two forcing terms.

$$E_\phi=k_\phi(\phi-\phi_o)^2 \text{ and } E_\psi=k_\psi(\psi-\psi_o)^2$$

A force constant of 1000Kcal/mol was used in order to force the angles ϕ and ψ to adopt conformation (ϕ_o,ψ_o), while minimising the total energy to relax all other degrees of freedom. The values of ϕ_o and ψ_o were incremented by steps of 10°, to produce the complete ϕ,ψ map. For the reduced and retroreduced residues, three maps were produced, with initial ω values of -60°, 180°, 60° and to account for the increased flexibility of this angle compared to an amide bond.

The resultant maps for compounds I-V are given in Figure 2. The energy contours of the ϕ,ψ maps are relative to the lowest energy point. The numbering of local minima and barriers is in the order of increasing energies.

Native Peptide (I).

There are three stable regions (local minima) in this flexible geometry map: (a) The C_7^{eq} conformation (inverse γ turn) is the lowest energy conformation. The extended conformation is not a local minimum, but is part of the wide C_7^{eq} low energy region. (b) Two distinct minima are observed in the right handed helix region. Although higher in energy than the C_7^{eq} conformation, these conformations create stable secondary structures due to the formation of inter-residue hydrogen bonding. (c) The C_7^{eq} conformation (classical γ turn) is a local minimum occasionally observed in proteins. (The left handed helix is not a stable minimum).

Reduced Peptide (II)

The C_7^{eq} region is destabilised since the hydrogen bond across the 7 membered ring cannot be formed since there is no C=O group at the appropriate position. Thus the energies of the C_7 and α_R regions are very close (within 0.1 Kcal) and the barrier is low (≈1 Kcal). A larger part of the conformational space is available to this modified residue, including regions on the right hand side of the map which are usually only favourable for D-amino acids. This means that this modification introduces increased flexibility and the residue can adopt nearly any conformation.

Reduced Peptide (III)

By replacing the C=O group with a CH_2 group a negligible intrinsic barrier to rotation of ψ for the native peptide is replaced by a three fold barrier (≈ 2.8 Kcal/mol). This results in three oval shaped minima, elongated in the ϕ direction in each half of the map ($\phi > O$ or $\phi < O$). Two of these minima are in regions close to the C_7^{eq} and α helical conformations of the native peptide. However the barrier between these conformations is higher for the reduced peptide. In addition a new local minimum was obtained which corresponds to an extended conformation. Another source of different conformational behaviour is that the high (≈20Kcal/mol) two-fold intrinsic barrier to rotation of ω for the native peptide is replaced with a low three-fold barrier (≈20Kcal/mol). Thus, conformations with $\omega=\approx\pm90°$ as well as $\omega = 180°$ are possible for some of the local minima.

The characteristic features of this modified peptide have important implications for incorporating the residue in secondary structures. First, it is possible to mimic all of the conformations of the native peptide since similar local minima exist for this modified residue. In addition, the existence of local minima for extended conformations allows the incorporation of this residue in a β sheet. However, care must be taken to include this modification only in an external strand with the reduced group pointing away from the sheet in order to avoid disruption of the hydrogen bonding in the sheet. It is also possible to introduce a 90° turn in the conformation of a peptide, similar to a disulphide bridge, since conformations with $\omega=\pm90°$ are favourable as well. However, the stability of $\omega=\pm90°$ and $\omega=180°$ conformations implies an unfavourable entropy of binding to the receptor or enzyme, since the enzyme will have to "select" the appropriate conformation for binding (It might be possible to overcome this by constrained structures - e.g. a 6 membered ring including the C_α N and C ensures $\omega=180°$).

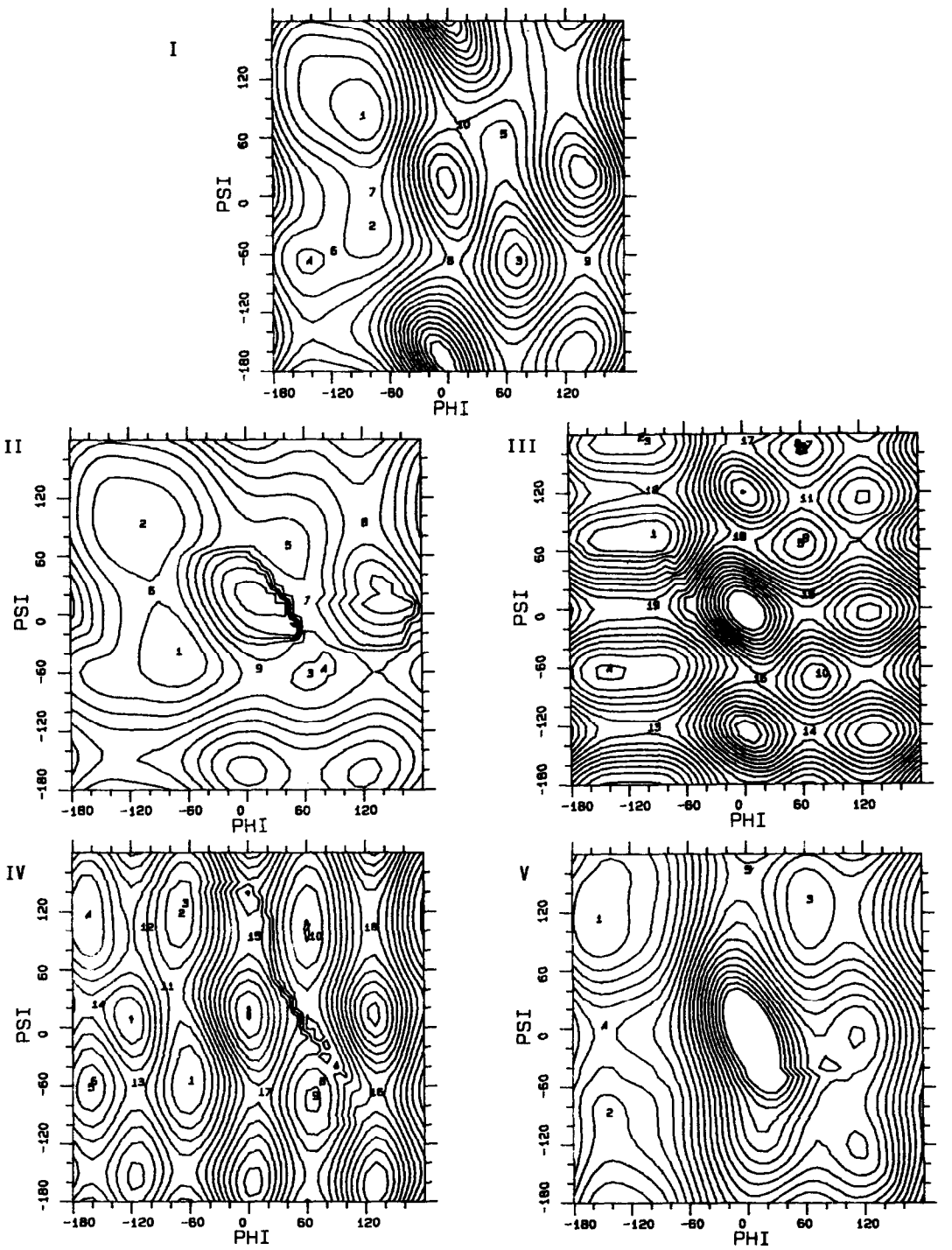

Figure 2

Retro-reduced Peptide (IV)

A negligible intrinsic barrier to rotation of ϕ for the native peptide is replaced by a three fold barrier ($\approx$ 2.8 Kcal/mol). This results in six oval shaped minima elongated in the ψ direction. A high ($\approx$20Kcal/mol) two-fold intrinsic barrier to rotation of ω for the native and the retro-inverso peptides is replaced with a low three fold barrier ($\approx$2Kcal/mol). Thus, some of the local minima conformations with $\omega = \approx \pm 90^{\circ}$ as well as $\omega = 180^{\circ}$ are possible. Again, it is possible to mimic the main conformations of the native peptide, as well as extended conformations and 90° turns as for the reduced peptide. Incorporation into secondary structures such as helices and sheets is complicated by mismatching of hydrogen bonding groups.

Retro-reduced Peptide (V)

The C_7^{eq} region is destabilised since the hydrogen bond across the 7 membered ring cannot be formed. (No C=O group at the appropriate position). The map quarter with (ϕ>O, ψ<O) is unstable due to the retro modification. (see ref. 6 for the conformational effects of the retro-inverso modification). This results in flexible regions in the left hand side of the map and high energy regions in the right hand side with one relatively high local minimum. Consequently, this residue can accommodate extended or α_R conformations, however regular α helices or β sheets will be disrupted due to a mismatch of the required hydrogen bonding groups. The C_7^{ax} conformation cannot be accommodated.

Thus we have described the major conformational effects of introducing reduced and retroreduced amide link surrogates. Full details of the various minima and barriers to conformational transitions will be given elsewhere.

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