Intermolecular stabilisation of the β-sheet conformation in dipeptides†

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Dipeptides are stabilised in the β-sheet conformation by three-point binding through hydrogen bonds to 3-amino-pyrazole binding sites.

Despite many efforts in recent years, only a few effective model compounds have been synthesised which stabilise the β-sheet structure in short peptides.[1] Most of them use intramolecular interactions between the peptide and its template facilitated by an interconnecting β-turn element.[1a–d] We present here, to the best of our knowledge, the first example of a purely intermolecular stabilisation of a β-sheet model, brought about by cooperative formation of hydrogen bonds between a dipeptide and a rigid template in a three-point binding mode. Our efforts are aimed at the development of artificial peptide receptors as well as the design of molecularly imprinted polymers for enantioselective peptide recognition.[2] Force-field calculations[3] show that five hydrogen bonds can be formed by interaction of two molecules of a 3-aminopyrazole with an *N*-acylated dipeptide ester, involving every peptidic hydrogen bond donor and acceptor available (Fig. 1). The top face of the peptide offers three binding sites, the bottom face only two. All hydrogen bonds can only be established if the peptide exists in the β-sheet conformation, as Fig. 1 demonstrates.

Furthermore, according to molecular modelling, larger peptides allow lining up of binding site molecules on both sides of the peptide strand, producing three hydrogen bonds per amino acid. Binding to large peptides should therefore reach high association constants.

When a dipeptide‡ was added to the suspension of an acylated aminopyrazole in $CDCl_3$, a clear solution was obtained and gave a first hint of interaction. The ^1H NMR spectra show large but markedly different downfield shifts for both peptide amide protons, further enhanced when the amount of aminopyrazole added was increased from 1 to 2 equiv. (up to 2 ppm). Observation of the heterocyclic NH-protons proved to be difficult, because these signals remain broad even in the complex.

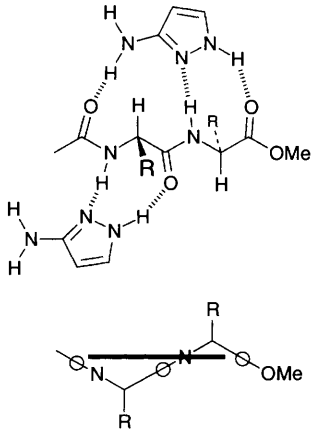
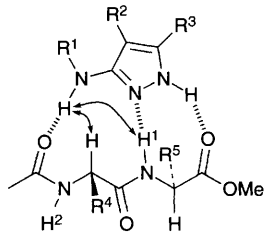


Fig. 1 Side and top view of the computer-calculated dipeptide–aminopyrazole complex;[3] when seen from above, the heterocycle is symbolised by a horizontal bar

We performed NMR titrations[5] of Ac-L-Val-L-Val-OMe **4** with pyrazole and 3-amino-5-methylpyrazole **2** (Fig. 2), measuring the complexation induced shifts of the amide protons (Fig. 3). Intramolecular NOE measurements allow distinction between NH(1) and NH(2), *i.e.* between the top and the bottom face of the peptide (Fig. 2). Pyrazole binds to both sides of the dipeptide with almost the same association constant (4.0 dm^3 mol^{-1}/3.8 dm^3 mol^{-1}) while aminopyrazole recognizes the top face four times stronger than the bottom face (10.2 dm^3 mol^{-1}/2.4 dm^3 mol^{-1}). We conclude that pyrazole binds to both sides in a two-point binding mode and does not distinguish between top and bottom face, while aminopyrazole forms three hydrogen bonds with the top face of the dipeptide. This is further substantiated by acylation of the amino group in **3**—the considerably stronger methacrylamide hydrogen bond discriminates much better between top and bottom face (80.0 dm^3 mol^{-1}/2.0 dm^3 mol^{-1}).

Job's method of continuous variations[6] confirmed the profound difference between the top face with 1 : 1 binding stoichiometry and the bottom face with 2 : 1 stoichiometry, indicating that the first receptor molecule is preferentially bound to the top face *via* stronger three-point binding while the second goes to the bottom face *via* two-point binding.

It is known that in a variable temperature NMR experiment in $CDCl_3$ free peptides below self-association concentration give



1 $R^1 = R^3 = H$; $R^2 = CO_2Et$
2 $R^1 = R^2 = H$; $R^3 = Me$
3 R^1 = methacryloyl; $R^2 = H$; $R^3 = Me$
4 $R^4 = R^5 = CHMe_2$
5 $R^4 = R^5 = Bn$
6 $R^4 = H$; $R^5 = CHMe_2$

Fig. 2 3-Aminopyrazole (**1–3**) and dipeptide derivatives (**4–6**)

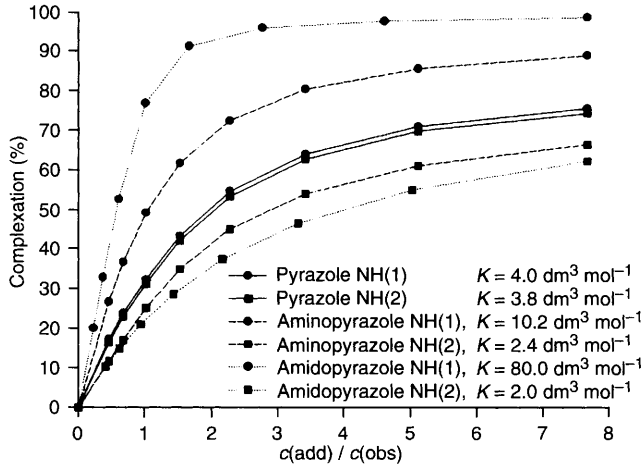


Fig. 3 ^1H NMR titration curves for the divaline complex with pyrazole, aminopyrazole **2** and amidopyrazole **3** (errors in *K* are ±5%)

only small values of *ca.* 3 ppb K^{-1} for the amide resonance.[7] We compared again divaline-derivative **4** and its complexes with pyrazole as well as with aminopyrazole **2**. Divaline dipeptide **4** alone, with 1 and with 2 equiv. of pyrazole shows temperature coefficients of 4, 10 and 15 ppb K^{-1}, respectively, with almost identical values for both amide protons of the dipeptide. By contrast, the complex with aminopyrazole gives 18 *vs.* 10 ppb K^{-1} for 1:1 and 23 *vs.* 14 ppb K^{-1} for 2:1 stoichiometry, the higher numbers counting for the top face, where three-point-binding is now possible. This demonstrates on one hand that very little self-association is occurring in the free dipeptide. On the other hand it proves that pyrazole binds to both faces exactly in the same way as aminopyrazole binds to the bottom face, *i.e.* in the two-point binding mode. Direct information about the peptide conformation can be drawn from Karplus analyses of the NH-α-CH coupling constants,[8] which correlate with the characteristic torsion angle θ. Our systematic examination gave the following results: addition of pyrazole, amino- or amido-pyrazole to dipeptides always produces sharper signals and increased ^3J values (in the range of 7.6–8.6 → 8.0–9.6 Hz), with aminopyrazole being consistently superior to pyrazole. Pyrazole leads to enhanced C-terminal torsion angles θ(1) in peptides by ≤7° [*e.g.* **5**: θ(1) = 151 → 158°, θ(2) = 149 → 152°], aminopyrazole **1** takes it even further to ≤11° [*e.g.* **4**: θ(1) = 157 → 168°, θ(2) = 155 →157°]; methacrylamidopyrazole **3** enlarges both torsion angles in **4** by 11 and 13°, respectively [θ(1) = 157 → 168°, θ(2) = 155 → 168°], although it only binds to the top face of the peptide!§ A further direct indication of induced conformational change within the peptide was found when small amounts of *N*-acylaminopyrazoles (*e.g.* **3**) were added to Ac-Gly-L-Val-OMe **6**, in which the glycine is conformationally free: the diastereotopic methylene protons immediately turn magnetically non-equivalent and at a 1:1 ratio the shift-difference reaches 0.25 ppm. This is only possible, if the amidopyrazole recognizes the third binding site, *i.e.* the *N*-acetyl group of the dipeptide. Now the Karplus equation[8b] produces a torsion angle θ(2) of 172° for the glycine residue which suggests an even more dramatic peptide folding than in the divaline case [θ(2) = 168°].

Intermolecular NOE effects would be especially conclusive, because they prove steric proximity of both complexation partners as well as their mutual orientation.[10] If the latter is determined by the strong interaction of the pyrazole nucleus with the C-terminal amino acid, then the critical interaction that has to be proven is the hydrogen bond between the pyrazole amide and the *N*-acetyl. According to molecular modelling[3] the distance between pyrazole amide and the *N*-terminal α-CH in the complex is *ca.* 280 pm while the C-terminal NH(1) is located *ca.* 380 pm away from it (see arrows in Fig. 2). We were able to detect small (0.5–1.5%) reciprocal NOE-effects for both signals.¶

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Footnotes

† A multimedia version of this article will be temporarily available from the CLIC page http://chemistry.rsc.org/rsc/clic.htm

‡ The dipeptides have been synthesized by DCC/HOBT coupling[4a] of *N*-acylamino acids and the respective amino acid ester hydrochlorides. Glycyl dipeptides were conveniently prepared by the mixed anhydride procedure[4b] using isobutyl chloroformate. Most aminopyrazoles were purchased and were regioselectively acylated at the amine functionality to strengthen the hydrogen bond of the third binding site.

§ It should be taken into account that already before complexation both dipeptides **4** and **5** exist to a considerable degree in the β-sheet conformation because of their sterically demanding substituents.[9] Therefore the relatively small rises in torsion angles represent indeed distinct effects.

¶ All samples were prepared under a dry argon atmosphere, exhaustively degassed by the freeze–thaw method, and sealed under vacuum. Nuclear Overhauser enhancements were obtained by saturation of the desired resonance during a preacquisition time set to 5 times the longest T_1 of the sample. Percent NOEs were calculated by setting the integral for the saturated resonance equal to −100 (inverted signal). The percent NOEs are reported as percentages of this inverted signal.

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