Novel disulfide-constrained pentapeptides as models for β-VIa turns in proteins

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Abstract The conformational behavior of cyclic peptides of the amino acid sequence Cys-Phe/Ala-Pro-Ala-Cys has been investigated through the combined use of molecular simulation methods and NMR experiments to find models for β-VIa turns of proteins. Both oxidized (cyclic) peptides and reduced (linear) forms were investigated. At least 95% of the cyclic peptides show a cis conformation of the Xaa-Pro bond in solution in DMSO or water, whereas all other peptide bonds are *trans*. Furthermore, we observed a hydrogen bond between the NH group of residue Ala[4] and the C = O group of residue Cys[1]. Both properties are indicative of β-VIa turns. After reduction of the disulfide bridge, the *all-trans* form of the peptide bonds predominates.

Key words: Cyclic peptides; Disulfide bridge; NMR; Solution structure; β-Turn; *cis*-Proline

1. Introduction

Low molecular weight polypeptides have often been used as models mimicking the process of protein folding and local folding patterns in protein structures [1–4]. Cyclic cystine containing peptides of limited ring size provide model systems for loop structures, helical structures and antiparallel β-sheet conformations [3,5,6]. 11- and 14-membered cyclic disulfides with one or two intervening amino acids between the two Cys residues linked by a disulfide bond can be used as models for γ-turn and β-turn conformations [1,5,6]. Proline exhibits a high positional preference for reverse turns in proteins [2,3]. A further unique property of prolyl residues is the ability to form both *cis* and *trans* peptide bonds in which the imide group is involved. The trans form of proline is observed in proteins in the *i*+1 position of type I or type II β-turns. In type VI turns, *cis*-proline occupies the *i*+2 position. The β-VIa turn shows a hydrogen bond between the NH group of residue *i*+3 and the C = O of residue *i* and forms a C_{10} conformation [3,7,8]. In proteins cis structured prolyl residues are observed in about 5–10% of all proline containing loops [9–11].

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Abbreviations: DIEA, *N*-ethyldiisopropylamine; Fmoc, 9-fluorenylmethyl-oxycarbonyl; HMQC, heteronuclear multiple-quantum coherence spectroscopy; NS, number of scans; ROESY, rotating frame nuclear Overhauser effect spectroscopy; SW, spectral width; TOCSY, total correlation spectroscopy; TOPPipU, 2-(2-oxo-1(2H)-pyridyl)-1.1.3.3-bis-pentamethyleneuroniumtetrafluoroborate; TPN, 3-(trimethylsilyl)-propionic acid-d_4-sodium salt.

The restriction in conformational degrees of freedom by cyclization increases the probability that experimental conformational analysis will identify predominant conformers in solution. We focus on the structures of peptides containing proline in either the *trans* conformation (open peptide) or in the *cis* conformation, depending on cyclization. Our aim was to find sequences for *cis*-proline containing cyclic peptides which mimic β-VI turns in solution and which are stabilized by a S–S bond. To find relevant sequences, we carried out a combined approach. Using force field methods, we first calculated the β-VI turn formation probabilities of different sequences. Second, we synthesized corresponding peptides which prefer *cis*-structured peptide bonds according to our calculations for structure determination by NMR.

In this report we describe the structure of pentapeptides of the sequence pattern Cys-Phe/Ala-Pro-Ala-Cys deduced from NMR experiments in solution and from energy calculations by simulated annealing/molecular dynamics calculations.

Chain reversals of this type seem to be useful as substrates of *cis-trans* prolyl isomerases [12,13] and in determining the tendencies of individual amino acids to form β-VIa turns [1].

2. Materials and methods

2.1. Energy calculations and molecular dynamic simulations (MD)

The aim of the energy calculations was to predict the probable occurrence of favourable *cis*-proline conformations for the different compounds which were taken into consideration. All energy calculations were carried out using the SYBYL Molecular Modelling package [14] which was implemented on a SGI Crimson workstation. The compounds were energy minimized to a convergence of energy gradient less than 0.001 kcal/mol·Å using the TRIPOS Force Field [15,16] included in the SYBYL MAXIMIN2 module. The partial atomic charges were computed using the Pullman method [17]. A distance independent dielectric constant $\varepsilon = 40$ was used in calculations to mimic the experimental conditions [18,19]. The manually built starting structures were minimized and used immediately as an input for simulated annealing dynamic runs. The molecules were heated to a temperature of 1000 K allowing equilibration and simulation for 20 ps followed by stepwise cooling in increments of 100 K up to 200 K. The simulation time for each step was 10 ps. This procedure was repeated 100 times. The resulting low energy conformations within the simulation at 200 K were stored and optimized by energy minimization. Thus, altogether for each peptide 200 low energy conformations (of course, not all were different) were obtained adopting *cis* as well as a *trans* conformation of proline residue. The resulting energy differences between the most stable *cis* and *trans* isomers are listed in Table 1. To estimate the expected percentage ratio of *cis*-proline conformations in solution, we performed a Boltzmann distribution analysis taking into consideration all low energy minimum *cis* and *trans* conformers of each compound we found. In order to reaching the best agreement of experimental and calculated data a restrained MD in in vacuo was performed, additionally, using

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41 interproton distance informations derived from ROESY spectra followed by unrestrained energy minimization.

2.2. Peptide synthesis

Peptides were synthesized using a standard protocol as described by Atherton and Sheppard [20]. The syntheses were carried out on a manual synthesizer using Cys(Trityl)-Wang resin, TOPPipU as condensing reagent and DIEA as base. All amino functions were protected by the Fmoc group. The sulphur group of cysteine was protected by the trityl group. Sulfhydryl groups were measured with a semiquantitative Ellman test [21]. The formation of the cystine bridge was obtained by air oxidation or by oxidation in 15% DMSO/water. The purity of the peptides were analysed by HPLC and mass spectrometry.

2.3. NMR studies

NMR studies were carried out on a Bruker AM 300 and AMX 600 NMR spectrometer using 25 mM samples in DMSO-d_6 and 90%H_2O/10%D_2O at 295 K. Several experiments were done at lower concentrations (about 4 mmol/l) to rule out intermolecular association. To obtain defined ionization states of the peptides in aqueous solution, the pH was adjusted to 3.5 by adding of minute amounts of HCl or NaOH. Solutions of the cationic form in DMSO-d_6 were obtained by lyophilizing the peptides from H_2O solutions and then dissolving the lyophilized materials in DMSO-d_6. All 2D NMR experiments, DQF-COSY, TOCSY, ROESY and HMQC have been aquired in the phase-sensitive mode with quadrature detection in both dimensions using time-proportional phase incrementation. TOCSY spectra were aquired with a 9 kHz MLEV-17 spinlock and 54 ms mixing time. ROESY spectra were recorded with a spinlock of 2.5 kHz and a mixing time of 100, 150 and 180 ms. ROESY crosspeaks were integrated by the AURELIA [22] software including offset correction according to Bull [23]. The ratio of *cis/trans* isomerism was determined by integration of the corresponding Cβ- and Cγ-signals in inverse gated decoupling spectra.

3. Results

3.1. Molecular modelling

The choice of length and sequence of peptides useful as models for β-VIa turns is limited by several prerequisites. In β-VI turns, proline occupies the i+2 position [3,7,8]. Consequently, peptides of the structure Cys-Pro-Cys must be longer than three residues for β-VI turns. Peptides with a high flexibility prefer the *trans* conformation [9–11,24]. Thus, we lengthened the peptide chain stepwise up to the number of residues which led to a preference for an *all-trans* peptide conformation also in cyclic form.

Table 1
Energy differences between the lowest energy of *cis* and *trans* form of predicted structures of several peptides

Peptide	ΔE *cis/trans* (kcal/mol)	% *cis*[a]	observed conformation and H-bonds; distance **NH** to **CO**[b]
c_{s-s} (**CPAC**)	−0.63	75	no H-bond observed
CPAC	−0.09	53	
c_{s-s} (CAPC)	−2.96	99	no H-bond observed
CAPC	−0.24	58	
c_{s-s} (**CFPAC**)	−6.18	100	C_{10}-conf. **CFPA**; 2.25 Å
CFPAC	1.10	11	
c_{s-s} (CAPAC)	−4.66	100	C_{10}-conf. **CAPA**; 2.23 Å
CAPAC	1.13	11	
c_{s-s} (CAFPAC)	1.72	5	C_7-conf. (γ-turn) [13] **FPA**; 2.14 Å

All energies were determined according to the description in section 2. c_{s-s}(CPAC) describes the disulfide bridged (oxidized) form of amino acid sequence of the type CPAC.
[a]Percentage ratio of *cis*-proline conformations estimated by Boltzmann distribution taking into consideration all local minimum *cis* and *trans* conformers of each compound we found. [b]In reduced form no H-bond was observed at all.

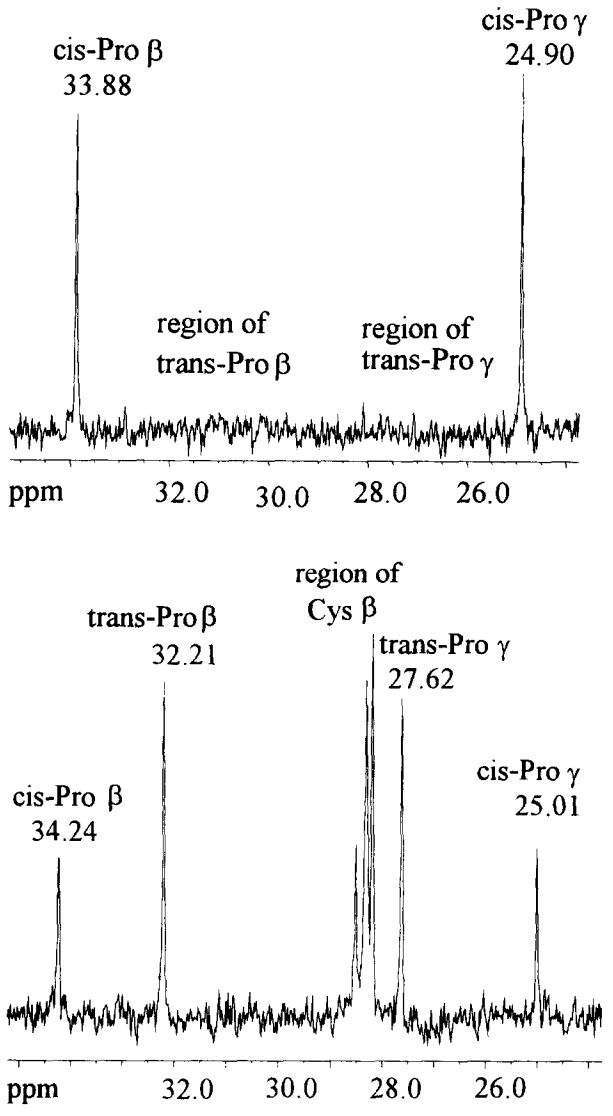

Fig. 1. Region of C^{β}- and C^{γ} proline resonance lines in the 300 MHz ^{13}C spectra of c_{s-s}(CFPAC) and the reduced form of CFPAC in H_2O/D_2O (90:10). The resonance signals of C^{β} of both cysteine residues in the top spectrum are low field shifted (39.2/43.2 ppm). Both spectra were calibrated with TPN. T = 295 K, pH 3.5.

We started with tetrapeptides with two different types of sequences (CPAC and CAPC) and end with hexapeptides which are highly flexible. To select the best candidat for our purpose, we use three criteria: (i) there must be a distinct H-bond in the peptide [3,7,8]; (ii) in cyclic form, the *cis* structure of Xaa-Pro must be more stable than *trans*; and (iii) reduced peptide should prefer an *all-trans* conformation.

For both tetrapeptides in the reduced state we predicted a relation between *cis* and *trans* near equilibrium, whereby the oxidized tetrapeptide seems to prefer more or less the *cis* conformation of the proline imide bond (Table 1). Cyclic peptides of the form c_{s-s}(CPXC) (X = different amino acids) were found by NMR experiments [1] to adopt a type I β-turn conformation centered at Pro, whereby all peptide bonds are mainly in the *trans* conformation. In molecular modelling studies done earlier [25] the existence of any H-bond of a *cis*-structured tetrapeptide could be ruled out. This agrees with our observa-

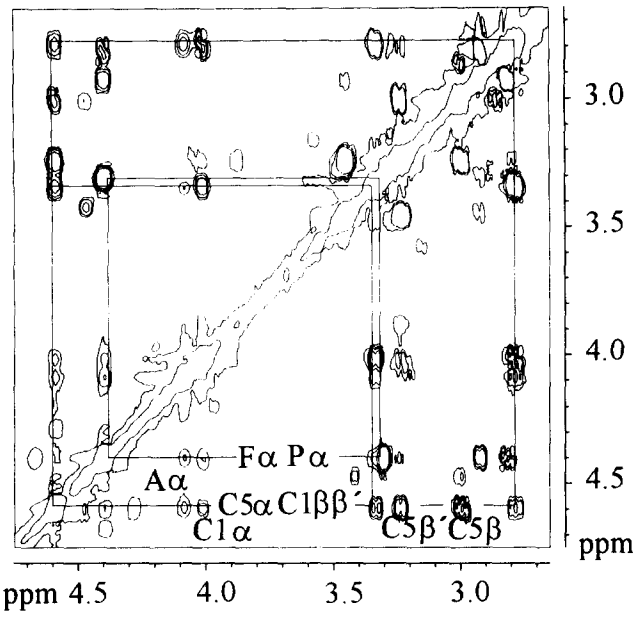

Fig. 2. H^{α}/H^{α} region of a 600 MHz ROESY spectrum of c_{s-s}(CFPAC) in DMSO-d_6 measured at 295 K. The spectrum was recorded with a mixing time of 180 ms and a spin lock power of 2.5 kHz. $T = 295$ K, pH 3.5.

tion. The reduced form of pentapeptides demonstrates lowest energy in the *all-trans* form. Comparing the most stable conformation and the estimated Boltzmann distribution to the different peptides, we found a strong preference for the *cis*-structured Xaa-Pro bond only for cyclic pentapeptides. This effect is pronounced for peptides containing phenylalanine at position $i+1$ (Table 1). The dynamic structure of the molecules became visible during the MD simulations and simulated annealing simulations. Several hydrogen bonded species of c_{s-s}(CXPAC) can be seen among the different structures which are mostly (more than 50%) closely related to β-VIa turns in the case of *cis*-proline. During simulation of the *all-trans* peptide, the formation of H-bonds is rarely observed.

Following these results of theoretical analyses and experimental results of other groups [1,9–11,24–26] we expected β-VIa turns with higher probability only in cyclic peptides of the structure c_{s-s}(CXPX'C). In consequence we focused our further considerations on pentapeptides.

3.2. NMR experiments

For cyclic peptides in both solvents, two different sets of resonance lines are found in ^{1}H spectra. These differences in spectra reflect the existence of *cis/trans* isomers of the Xaa-Pro peptide bond. In ^{13}C spectra, the resonances of different proline isomers can be easily identified considering the C^{β} and C^{γ} shift values in the proline side chain (Fig. 1). The chemical shift values, 33.9 ppm for C^{β} and 24.9 ppm for C^{γ} of proline, respectively, and the differences between both signals, 9.0 ppm, is characteristic for *cis*-proline isomers in water. The corresponding resonances for the trans form are not visible in oxidized peptides, but can be observed in the open form (32.2 ppm for C^{β} and 27.6 ppm for C^{γ}).

Furthermore, *cis* and *trans* isomers give different crosspeaks in ROESY spectra between the Xaa$^2H^{\alpha}$ atom and the ProH^{α}/ProH^{δ} (Fig. 2). A short H^{α}–H^{α} distance of the second amino acid (Phe or Ala) and Pro^3 is observed in *cis*-structured peptides: the resulting intense cross peak is seen only in ROESY spectra of disulfide bridged peptides. Regardless of solvent, at least 95% of both cyclic pentapeptides investigated show a *cis* Xaa-Pro imide bond.

Several strong NOEs were observed between Ala^{4}NH and hydrogens of the pyrrolidine ring. Additionally, NOEs were also estimated between Ala$^4H^{\alpha}$ and Cys$^1H^{\beta}$ proton and Ala^{4}NH and Phe$^2H^{\alpha}$/Ala$^2H^{\alpha}$. This pattern of NOEs and the small temperature dependence of the chemical shift of Ala^{4}NH (see below) can be explained by the existence of a β-VIa turn in both peptides, with Pro at position $i+2$ of the turn and with a hydrogen bond between the carbonyl of Cys^1 and NH of Ala^4. All other peptide bonds are in *trans* conformation, as there are no strong H^{α}/H^{α} crosspeaks in the ROESY spectra other than that of Ala-Pro or Phe-Pro.

Besides the phenylalanine side chain both cyclic peptides show nearly identical structures (Fig. 3). The temperature coefficients of the amide protons of Ala^4 are small ($1.7 \cdot 10^{-3}$ ppm/K

Table 2
Comparison of dihedral angles calculated from coupling constants with theoretically predicted dihedral angles for $c_{s\ s}$(CFPAC) and $c_{s\ s}$(CAPAC) in *cis* form.

Amino acid	NMR[a]	Molecular modelling[b]			β-VIa turn [7]	
	ϕ	ϕ	ψ	χ^1	ϕ	ψ
$c_{s\ s}$(CFPAC)						
Cys^1	–	–	−63 (−61)	−69; −142 (−69; −147)		
Phe^2	−83; −157	−37 (−47)	128 (125)	−178 (−178)	−60	120
Pro^3	–	−58 (−72)	−35 (−21)	–	−90	0
Ala^4	37; 83; −74; **−166**	−161 (−162)	159 (159)	–		
Cys^5	**−90**; −150	−76 (−80)	–	−54 (−55)		
c_{s-s}(CAPAC)						
Cys^1	–	–	−63 (−62)	−67; −141 (−68; −143)		
Ala^2	−86; −154	−33 (−41)	122 (121)	–	−60	120
Pro^3	–	−72 (−72)	−23 (−23)	–	−90	0
Ala^4	38; 82; −75; **−165**	−162 (−163)	157 (160)	–		
Cys^5	**−93**; −147	−78 (−81)	–	−54 (−55)		

[a]The modified Karplus equation $^3J_{NH^{\alpha}} = 6.4\cos^2(\phi - 60) - 1.4\cos(\phi - 60) + 1.9$ was used for the calculation of the dihedral angle ϕ. We note all possible solutions in the table. The plausible solution is marked. Coupling constants were measured in DMSO at 295 K. [b]The first value listed is for an experimentally determined low energy conformation without NOE restraints and the value in parentheses is for an averaged conformation of NOE restrained MD.

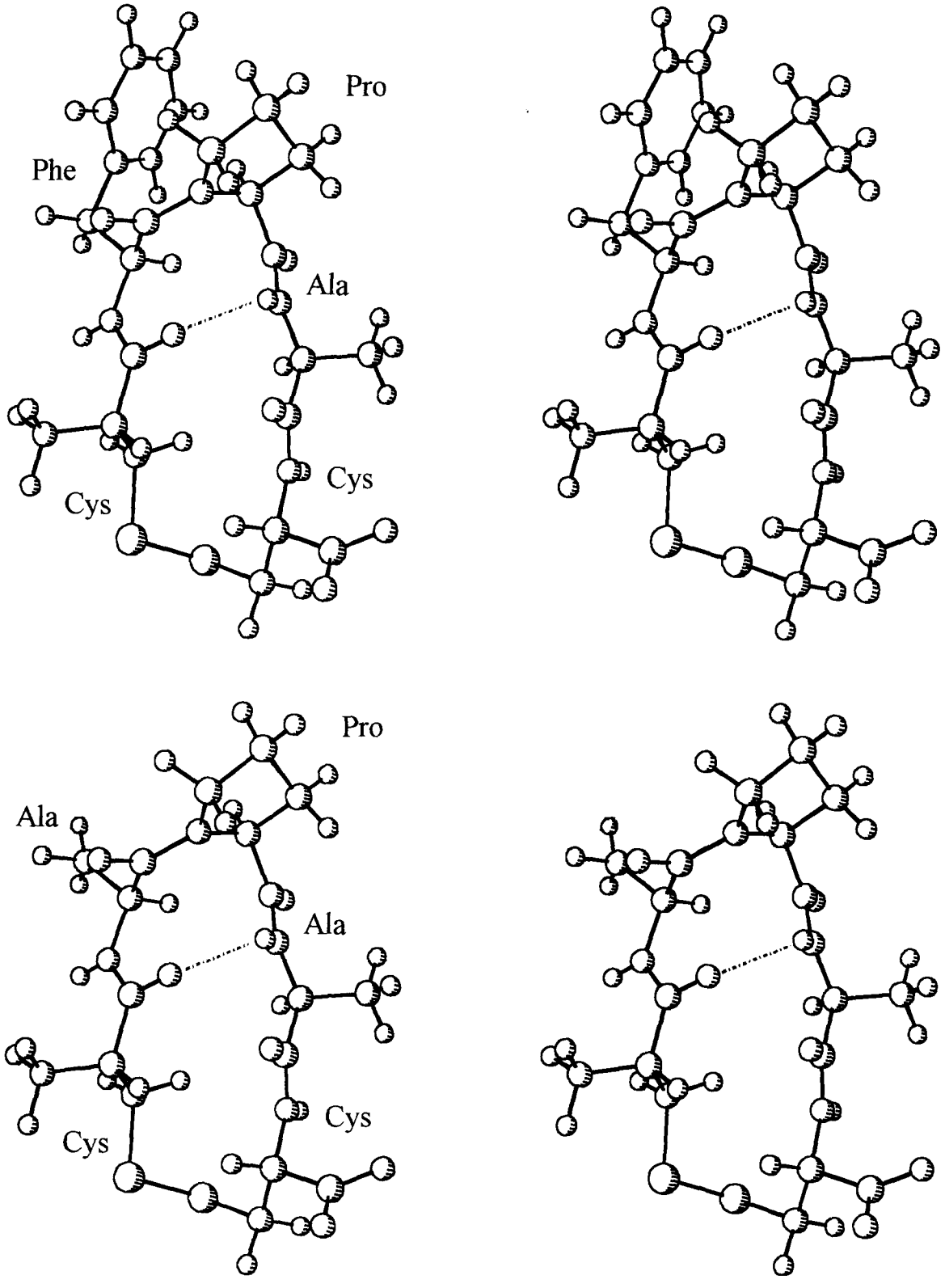

Fig. 3. Stereoplots of $c_{s\text{-}s}$(CFPAC) (top) and $c_{s\text{-}s}$(CAPAC) (bottom). H-Bonds are indicated by a dashed line.

and $1.9 \cdot 10^{-3}$ ppm/K in DMSO). These data suggest that either the Ala^4NH of both peptides is involved in a hydrogen bond or the Ala^4NH is surrounded by hydrophobic residues shielding it from the solvent. Furthermore, NH of Ala^4 shows a small $^3J_{NH}\alpha$ coupling constant in the range of 4.8–6.1 Hz. The analysis of the calculated torsion angles ϕ, ψ and the $^3J_{NH}\alpha$ coupling constants supports the view that a β-VIa turn conformation exists in both peptides (Table 2). This observation is valid for

residues can be derived. In the case of reduced CAPAC, we estimate a *cis/trans* ratio of 1:15. Surprisingly, in the reduced form of CFPAC we estimate about 30% *cis* form of the Phe-Pro peptide bond. When the temperature was increased by about 30 K, we observed an increase in the cis-content of about 3–5%. Due to the high content of both *cis* and *trans* conformers, we are able to compare NMR data of the two. Remarkable differences are observed in chemical shifts of proline protons. The proton resonances of reduced CFPAC, in which the Phe-Pro peptide bond is in the *cis* conformation, are very similar to those of the cyclic form, indicating that the prolines have similar surroundings in both structures. We assume that the phenyl ring and the pyrrolidine ring form a sandwich like structure in the reduced peptide when there is a *cis*-structured peptide bond between phenylalanine and proline. The *cis* conformation is probable stabilized by additional hydrophobic interactions.

4. Discussion

The planar peptide bond –N–C(O)– in proteins is known to exist overwhelmingly in the *trans* conformation. Out of all amide bonds in the Brookhaven Protein Data Bank, only 0.05% are *cis* while 6–8% of imide bonds (Xaa-Pro) are *cis* [9,10]. Interestingly, all *cis*-structured peptide bonds are in loop conformations [9]. There are several models for β-turn conformations whereby peptides of different lengths and peptidomimetica are used [2,9,27]. The four residue β-turns show a hydrogen bond formed between the main chain NH-group of the fourth amino acid (in our case Ala^4) and C = O of the first residue (Cys^1 resulting in a C_{10} conformation). On the basis of the classification of Richardson [7] proline containing loops mainly adopt β-turns of type I and II if proline is in position two and the imide bond is in the *trans* conformation. A *cis*-proline is found in the third position of type VI loops (*cis*-proline turn) [2]. Considering several properties of β-VI turns (Table 2), we suggest that the cyclic pentapeptides of the structure $c_{s\ s}$ (CXPAC) mimic the β-VIa turns of proteins showing a dihedral angle ϕ_{i+2} (Pro) near −90° (−75°) and H-bond between NH_{i+3} and CO_i. The NMR structure determination of $c_{s\ s}$ (CAPAC) and c_{s-s}(CFPAC) confirm our expectations, based on molecular dynamic calculations, that the cyclic pentapeptides examined here have a high probability of forming type VIa β-turns with *cis*-structured peptide bonds of prolyl residues.

The reduced pentapeptide gives unambiguous signals showing that the population of *cis* isomers becomes less than 5% (CAPAC) or 30% (CFPAC). Therefore we observe a nearly complete transition from *cis*- to *trans*-proline isomers after reduction of the pentapeptide. All minor peaks in the spectrum can be attributed to molecules having the Xaa-Pro peptide bond in the cis conformation. The relation of these peaks to those assigned to the trans form show that 5% (±3%) are in *cis* in CAPAC. This corresponds to a free energy difference between the two isomers of about 2 kcal/mol, consistent with other findings in literature [12,13,24]. Of course, the *cis/trans* equilibrium of the Xaa-Pro bond also depends on the pH of the solution and the electrostatic interactions, earlier discussed in detail by Grathwohl and Wüthrich [26]. The population of *cis* isomers increases by the transition from cationic to zwitterionic ionization state.

A comparison of theoretical and experimental results shows that even though no explicit free energy could be calculated, the agreement between experiment and theory is good enough to support planning experiments. Despite the lack of explicit terms for hydrophobic interaction and entropy and an incomplete description of coulombic interactions [18,19], the energy calculation correctly predicts the trend of stability of the conformers as determined by NMR experiments. This result seems to be due to the dominance of covalent constraints in our small disulfide bridged peptides. Furthermore all compounds considered are quite similar in structure. Accordingly the systematic errors of energy calculations are greatly compensated comparing only homologous peptides. In our molecular modelling study, we consistently predict an overly high content of the *cis* Xaa-Pro conformation, especially for reduced peptides. We believe this is caused by an overestimation of coulombic interactions which is apparently strengthened by the shorter C^α-distance of the *cis*-structured peptide bond. Several recent studies [28] of tetrapeptides of the structure $c_{s\ s}$(CXYC) suggest that the disulfide bond in the 14-membered ring forces the adoption of a 4→1 intramolecular hydrogen bond observed in β-turn conformations, whereby all peptide bonds are in the *trans* conformation. Hexapeptides of the analogous structure (c_{s-s} (CX_4C)) adopt an antiparallel β-sheet conformation with an *all-trans* backbone conformation [29]. The results of systematic studies of small disulfide loops containing a limited number (n = 1–5) of intervening amino acids between the two linked cysteine residues [30] show that ring closure is generally favored in disulfide bridged peptides with even values of n relative to loops with odd n values. This finding is consistent with our result that there are covalent constraints in the 17-membered ring. This 'ring tension' shifts the equilibrium between *cis* and *trans* forms of Xaa-Pro from about 1:20 (reduced CAPAC) to 19:1 ($c_{s\ s}$ (CAPAC)), corresponding to an energy difference of less than 4 kcal/mol. This value is even less than the energy difference between the *cis* and *trans* forms of amide bonds in proteins [9]. We therefore assume that oxidized pentapeptides without proline residues adopt an *all-trans* conformation.

The class of peptides considered here may also be useful in additional investigations, e.g. analysis of the substrate specifities of prolyl-isomerases and prolyl-dependent peptidases and for conformational and energetic investigations of loop structures in proteins.

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both peptides and both solvents. Therefore we assume that the stability of the *cis*-conformation is mainly a result of covalent constraints and not of noncovalent forces such as hydrophobic or coulombic interactions.

The determination of the side chain orientation of phenylalanine using NOEs (Table 3) and homonuclear coupling constants results in a value of about 180° for χ_1, χ_2 cannot be determined, but in a low energy rotamer χ_2 adopts a value of about −60°. This combination of torsion angles χ_1 and χ_2 allows the sandwich like parallel alignment of the phenyl ring relative to the pyrrolidine ring. Further evidence for the close contact of both rings in peptides with *cis*-proline arises from the chemical shift values of the ProH$^{\alpha}$ resonances which show a significant highfield shift (3.30 ppm). The highfield shift of proline H$^{\beta}$ proR at 1 ppm highfield can be explained by close contact of this proton to the anisotropic electron system in the phenylalanine ring. As a driving force for such a turn conformation, Kessler and coworker propose the existence of hydrophobic interactions between the phenyl ring and the proline ring [2].

The strength of NOEs served as the basis for the determination of the major conformation in solution of the oxidized peptide. We compared all distances between pairs of protons calculated in low energy conformations (up to an energy of 7 kcal/mol higher than the most stable one) by force field methods with estimated NOEs. In this way, we determined that the conformations generally reflect the experimental results (Table 2). In the case of c_{s-s}(CXPAC), the experimentally determined solution conformation is a member of the low energy conformations within the range of 3 kcal/mol from the most stable conformation. These structures are represented in Fig. 3. Additionally, the dihedral angles calculated on the basis of the Karplus equations were taken into consideration. Parallel to this procedure we carried out restrained MD calculations which support the results described here (Tables 2 and 3).

NMR data of reduced peptides were determined only in water because we notice a slow conversion to cyclic forms in DMSO caused by oxidation. Due to the high flexibility of the reduced forms of peptides, the conformational investigations permit only few conclusions. All NMR parameters suggest an equilibrium between several conformations. All resonance lines lie in the expected region. The temperature dependence of amide proton resonances does not indicate hydrogen bonds or shielding of NH. All values are about $7 \cdot 10^{-3}$ ppm/K. The $^3J_{NH}\alpha$ coupling constants vary in the range of ±2 Hz. This value cannot be converted meaningfully into dihedral angles, as it represents an average of coupling constants of different conformations.

From C$^{\beta}$ and C$^{\gamma}$ resonances the *cis*/*trans* ratio of proline

Table 3
Comparison of the experimental (ROESY in DMSO-d_6) and calculated (restrained MD) interproton distances of both cyclic pentapeptides $c_{s\ s}$ (CFPAC) and c_{s-s}(CAPAC)

Proton pairs		$c_{s\ s}$(CFPAC)		c_{s-s}(CAPAC)	
		NMR $_rNOE$	MD [Å]	NMR $_rNOE$	MD [Å]
Cys^{1}Hα	Xaa^{2}NH	2.89	3.05	2.96	3.25
Cys^{1}H$^{\beta}$(R)	Ala^{4}H$^{\alpha}$	3.13	2.96	3.15	2.91
Cys^{1}H$^{\beta}$(R)	Cys^{5}NH	3.05	2.90	3.27	2.27
Cys^{1}H$^{\beta}$(R)	Cys^{5}H$^{\alpha}$	2.96	2.96	3.11	3.08
Xaa^{2}NH	Xaa^{2}H$^{\alpha}$	2.64	2.60	2.66	2.60
Xaa^{2}NH	Xaa^{2}H$^{\beta}$ (S)	2.42	2.50	H$^{\beta}$ 2.79	2.79
Xaa^{2}NH	Xaa^{2}H$^{\beta'}$ (R)	2.76	2.83	–	–
Xaa^{2}H$^{\alpha}$	Pro^{3}H$^{\alpha}$	2.12	2.12	2.16	2.40
Xaa^{2}H$^{\alpha}$	Ala^{4}NH	3.10	3.30	3.11	3.42
Phe^{2}H$^{\delta'}$	Pro^{3}H$^{\alpha}$	2.85	3.22	–	–
Phe^{2}H$^{\delta'}$	Pro^{3}H$^{\beta}$ (S)	3.58	3.40	–	–
Phe^{2}H$^{\delta'}$	Pro^{3}H$^{\gamma,\gamma'}$	3.80	3.78	–	–
Phe^{2}H$^{\delta'}$	Pro^{3}H$^{\delta}$	2.98	3.00	–	–
Phe^{2}H$^{\varepsilon}$	Pro^{3}H$^{\alpha}$	3.53	3.60	–	–
Phe^{2}H$^{\varepsilon}$	Pro^{3}H$^{\beta}$ (S)	3.80	3.91	–	–
Phe^{2}H$^{\varepsilon}$	Pro^{3}H$^{\gamma,\gamma'}$	3.62	3.64	–	–
Phe^{2}H$^{\varepsilon}$	Pro^{3}H$^{\delta}$	3.72	3.80	–	–
Pro^{3}H$^{\alpha}$	Pro^{3}H$^{\beta}$ (S)	2.33	2.43	2.70	2.98
Pro^{3}H$^{\alpha}$	Pro^{3}H$^{\beta'}$ (R)	2.74	2.74	2.30	2.45
Pro^{3}H$^{\alpha}$	Ala^{4}NH	2.53	2.65	2.65	2.66
Pro^{3}H$^{\beta'}$ (R)	Ala^{4}NH	3.39	3.39	3.20	3.11
Pro^{3}H$^{\delta'}$	Ala^{4}NH	3.15	3.15	2.95	2.60
Ala^{4}NH	Ala^{4}H$^{\alpha}$	2.60	2.87	2.51	2.84
Ala^{4}H$^{\alpha}$	Cys^{5}NH	2.24	2.40	2.26	2.40
Cys^{5}NH	Cys^{5}H$^{\alpha}$	2.86	2.90	2.88	2.95
Cys^{5}NH	Cys^{5}H$^{\beta}$ (R)	2.54	2.58	2.67	2.58
Cys^{5}NH	Cys^{5}H$^{\beta'}$ (S)	2.78	2.78	2.83	2.73
Cys^{5}H$^{\alpha}$	Cys^{5}H$^{\beta}$ (R)	2.72	2.97	2.70	2.96
Cys^{5}H$^{\alpha}$	Cys^{5}H$^{\beta'}$ (S)	2.38	2.46	2.25	2.45

Xaa^2 means amino acid in position 2, in c_{s-s}(CFPAC) Phe, in $c_{s\ s}$(CAPAC) Ala.

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