SALT BRIDGES IN MODEL PEPTIDES

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Dedicated to the memory of Dr Karel Bláha.

A group of synthetic peptides including Boc-Lys-Phe-X-Y, X = Ala (*I*, *III*) or Thr (*II*), Y = = Pro (*I*, *II*) or Ala (*III*) was studied by means of ^{1}H NMR spectroscopy and theoretical conformational analysis. Compound *I* in DMSO shows two conformers with the *trans*- and *cis*-configuration of the peptide bond Ala-Pro. The salt bridge between the Lys ε-amino group and the C-terminal carboxyl is featured by magnetic nonequivalence of the Lys $C^{\varepsilon}H_2$ protons. The space structure of *I* and *II* was found to possess a salt bridge fixed by an unusual turn in the chain formed by the Lys side chain and the C-terminal dipeptide with the *trans*-peptide bond X-Pro. Since a stable ionic bond in *III* and in the *cis*-conformer of *I* has not been observed, its contribution to stabilization of the space structure of the peptides in DMSO appears rather small.

Electrostatic interaction between ionic pairs is a major factor contributing to the structure and function of peptides/proteins. The role of such interactions in the stabilization of spatial structure in oligopeptides and proteins by way of salt bridges formed between oppositely charged groups has been a subject for discussion in several theoretical and experimental papers (see, e.g.[1,2]).

The practical application of NMR spectroscopy to space structure studies has frequently raised the issue of ionic bond identification. The presence of salt bridges in such peptides as bradykinin, angiotensin, tuftsin, etc. has been proved or rejected with varying degree of ambiguity[3-5]. At present it can be only claimed that salt bridges are absent in small oligopeptides in aqueous solution or, at least, they are not responsible for the stabilization of their steric structure. In aqueous environment, charged groups in peptides undergo hydration, therefore ionic interactions are relatively weak. However, in aprotic solvents ionized groups can form an intramolecular salt bridge, which substantially contributes to the stability of the preferential space structure[3]. Interaction of this kind involving the charged side chains has been noted in DMSO, for the tetrapeptide in which the central conformationally restricted residues Pro and Aib were responsible for the β-turn in the peptide back- bone[6].

This work is an attempt to investigate synthetic peptide compounds as models useful for studying intramolecular salt bridges between the positively charged side chain and backbone carboxyl and elucidate their role in the stabilization of the space structure of oligopeptides. To this end, we synthesized and examined by means of NMR spectroscopy and theoretical conformational analysis a group of Boc-Lys-Phe-X-Y-COO^- tetrapeptides carrying Ala or Thr in position X and Pro or Ala in position Y.

EXPERIMENTAL

Synthesis: The compounds in question were synthesized by the classical methods of peptide chemistry with pentafluorophenyl esters of protected amino acids used as activated component at all stages of the coupling procedure.

^{1}H NMR *spectroscopy:* All NMR spectra were recorded on a Bruker WM-360 spectrometer equipped with an Aspect 2 000 computer and a satellite station with an Aspect 1 000 computer. The software used to obtain two-dimensional NMR spectra was from Bruker Instruments, version DISNMR 86 01 01·0. For NMR experiments c. 0·02M solutions of peptides were prepared in $(CD_3)_2SO$ (Izotop, U.S.S.R.) without degassing.

COSY spectra were obtained with the pulse sequence $90° - t_1 - 90° - t_2$ and ROESY spectra with the pulse sequence $90° - t_1 - (\beta - \tau)_n - t_2$ (ref.[7]) by using the mixing sequence $(\beta\tau)_n$ with $\beta = 3$ μs (32·5°), $\tau = 30$ μs and $n = 6\,000$. The total mixing time was 198 ms. The 90° pulse length was 8·3 μs. The carrier frequency was positioned at 5·31 ppm. For both spectra, quadrature detection in the F1 dimension was achieved by the TPPI method[8]. The spectral width in the F2 dimension was 4 000 Hz (2 048 data pts) and 96 transients were collected for each of 256 increments with a relaxation delay 1·6 s between successive transients. Zero filling to 1 K in t_1 was applied. The data were multiplied by phase-shifted window functions: sine bell-squared phase-shifted by $\pi/4$ along t_2 and sine bell phase-shifted by $\pi/4$ along t_1 prior to Fourier transformation.

The 1D-ROESY experiment was performed in the difference mode by the pulse sequence $(180°)\text{-}90°\text{-}(\beta - \tau)_n - t_2$. The selective 180° pulse was 40 ms for decoupler with DP = 35 L and was applied to the centre of the proton line of interest. 128 transients were acquired with the selective pulse on resonance frequency of the spin to be saturated. Then the same transients were recorded with the off-resonance selective pulse and both spectra were subtracted.

Energy calculations: Calculations of conformational energy for the N-acetyl derivatives of tetrapeptides *I* and *II* (without taking account of the N-terminal Boc group) were performed on an HP-1000 computer using the standard peptide geometry and potential functions described by Momany et al.[9]. All peptide groups were regarded as planar, the peptide bonds X-Pro were both in the *trans*- and *cis*-configurations. The pyrrolidine ring of proline was fixed in the "*down*" conformation[9]. The initial backbone conformations for energy minimization were obtained by combining local minima[10] for individual residues. Initial conformations for the side chains were chosen by varying each dihedral angle χ by 30° (ref.[11]). The lysine ε-amino group and the C-terminal carboxyl were assumed ionized.

The selection of conformations permitting close spatial arrangement of ionized groups was carried out by preliminary energy minimization with the dielectric constant value $\varepsilon = 2$. Thereby, the overestimated contribution of electrostatic interactions appears sufficient to overcome the small barriers of nonbonded interactions and torsional potentials in the course of minimization,

which allows the selection of conformers with intramolecular salt bridges without systematic scanning the rotamers for the lysine side chain. Energy calculations both for structures with salt bridges and for backbone conformers ruling out close location of ionized groups were performed at $\varepsilon = 47$ corresponding to DMSO. This approximation apparently accounts for the underestimation of contribution provided by electrostatic interactions to the stabilization of salt bridges, i.e. closely located ionic groups may lead to a reduction in the effective dielectric permeability of their microenvironment.

RESULTS AND DISCUSSION

^{1}H NMR *Spectroscopy*

Fig. 1 depicts ^{1}H NMR spectra (360 MHz) for the tetrapeptides Boc-Lys-Phe-Ala-Pro (*I*) and Boc-Lys-Phe-Thr-Pro (*II*) in DMSO. Two sets of resonance signals are observed in the spectrum of tetrapeptide *I*, corresponding to the *trans*- and *cis*--conformers of the peptide bond Ala-Pro. The use of two-dimensional correlation spectra (COSY) allowed a conclusive assignment to be made of most resonances for the two conformers of tetrapeptide *I*. However, the assignment of signals for the strongly bonded spin systems in the Lys and Pro side chains is not feasible at 360 MHz even with recourse to the two-dimensional methodology of relayed correlation spectroscopy. Identification of signals for the lysine $C^\varepsilon H_2$ protons could be carried out by integrating the corresponding regions of the tetrapeptide *I* spectrum owing to differences in the population of the two conformers. The major conformer was found to possess nonequivalent Lys $C^\varepsilon H_2$ protons (δ 2·70 and 2·89 ppm), the same protons being equivalent in the minor conformer (δ 2·67 ppm).

Resonance signals in the ^{1}H NMR spectra of tetrapeptide *II*, Boc-Lys-Phe-Ala-Ala (*III*) and Boc-Lys(Z)-Phe-Ala-Pro-ONb (*IV*) were assigned by the double resonance method. The chemical shifts of NH protons, $^3J(HNC^\alpha H)$ values and temperature coefficients of the chemical shifts for amide protons of compounds *I*–*IV* are summarized in Table I. Only one conformer characterized by considerable nonequivalence of the Lys $C^\varepsilon H_2$ protons (δ 2·65 and 2·84 ppm) is noted for tetrapeptide *II*. The same protons in tetrapeptide *III* are practically equivalent (Fig. 2).

The $C^\varepsilon H_2$ signals in all lysine derivatives are commonly equivalent and occur in the form of a triplet. Only limited mobility of the lysine side chain caused by a hydrogen bond in the salt bridge can make the specific rotamers of the C^ε—N bond more preferable[6]. This allows to suggest that the nonequivalence of Lys ε-protons in tetrapeptide *II* and in the major conformer of tetrapeptide *I* can be explained by the formation of a salt bridge between the lysine ε-amino group and the C-terminal carboxyl.

The major source of structural information from NMR studies of peptides is the proton two-dimensional nuclear Overhauser enhancement experiment (NOESY)[12]. However, in medium size molecules, such as peptides with 5–15 residues, cross-peak

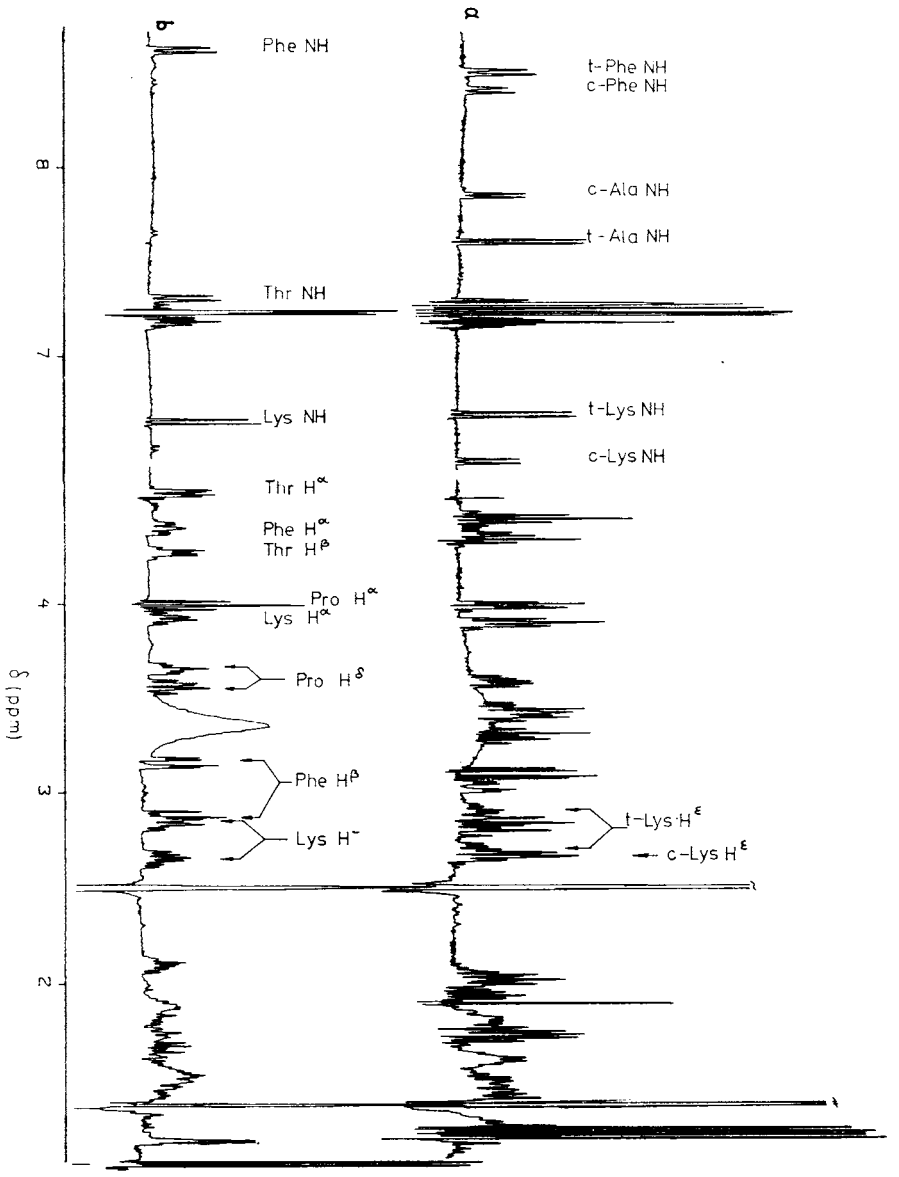

FIG. 1

The 360 MHz ^{1}H NMR spectra of Boc-Lys-Phe-Ala-Pro (*a*) and Boc-Lys-Phe-Thr-Pro (*b*) in $(CD_3)_2SO$ at 303 K

intensities are close to zero, because the motional correlation time τ_c in most solvents satisfies the condition $\omega\tau_c \simeq 1$. Recently, an alternative to the standard NOESY experiment has been proposed in the form of the rotating frame Overhauser enhancement spectroscopy (ROESY)[13,14]. In the rotating frame the NOE during the period of spin locking is always positive and increases with τ_c.

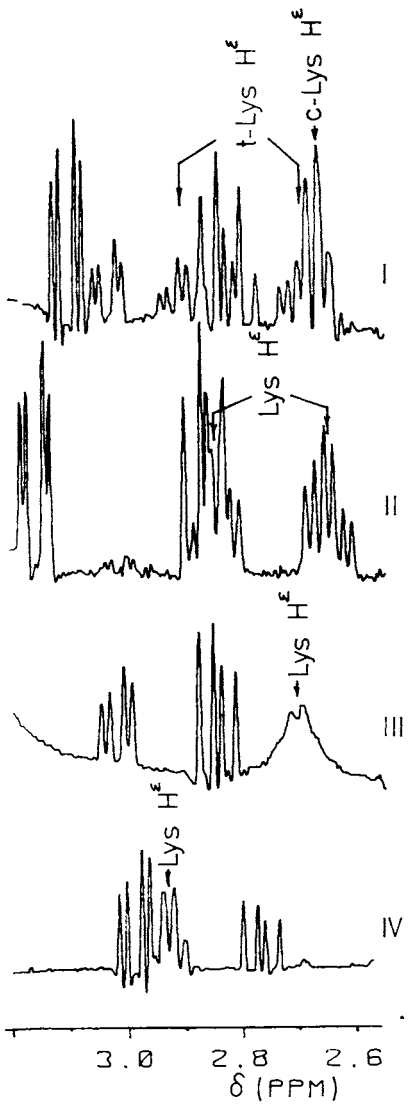

FIG. 2

A region of the ^{1}H spectrum of the $C^\varepsilon H_2$ Lys protons for peptides *I*—*IV* in $(CD_3)_2SO$ at 303 K

The pulse scheme of the ROESY experiment is shown in Fig. 3. At the end of the evolution period, a strong rf field is applied for a period τ_m. During the mixing time τ_m, four different types of magnetization transfer can occur: cross-relaxation along the effective rf field vector similar to spin exchange along the $\pm z$ axis in the

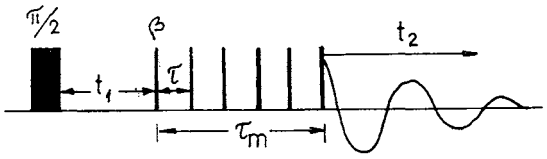

FIG. 3

Pulse sequence for 2 D ROESY. Mixing sequence $\tau_m = (\beta\tau)_{6000} = 198$ ms

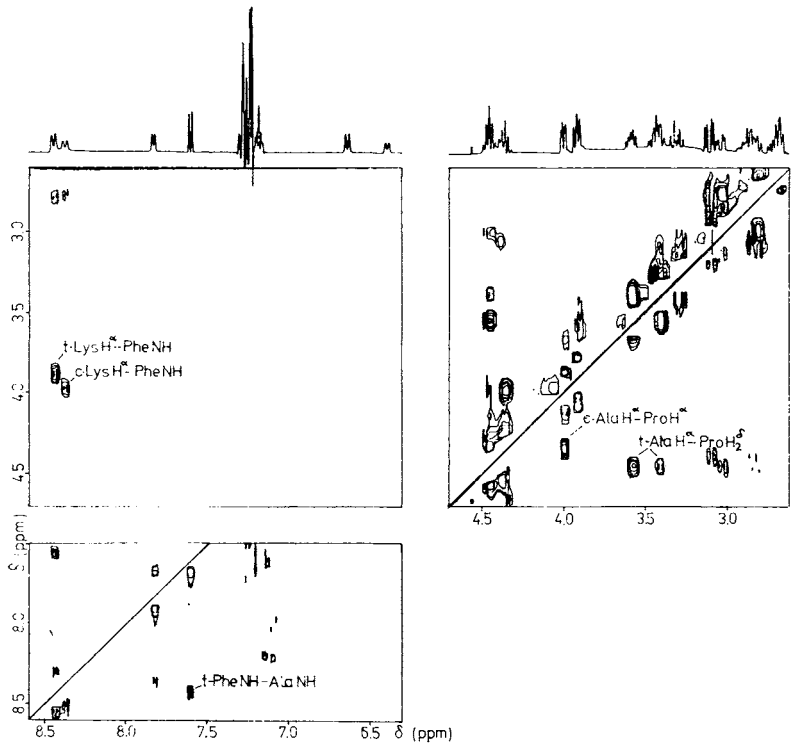

FIG. 4

2 D ROESY spectrum of Boc-Lys-Phe-Ala-Pro in $(CD_3)_2SO$ at 303 K. Only negative levels are plotted

conventional NOE experiment; the Hartman–Hahn polarization; chemical exchange; COSY transfer. NOE peaks in the ROESY experiment have the opposite signs with respect to the diagonal peaks and they can be differentiated from the chemical exchange and Hartman–Hahn polarization peaks[14,15]. COSY transfer peaks have an antiphase character[14]. Mixing in the rotating frame can be achieved by various methods[7,13], but we used a repetitive pulse sequence with the period $(\beta - \tau)$, which minimized magnetization transfer by Hartman–Hahn polarization[7].

Fig. 4 displays three regions of a ROESY spectrum for tetrapeptide *I*. In this contour plot only resonances that have the opposite sign, as compared to the diagonal resonances, are displayed, i.e. resonances that are due to transverse NOEs. An intensive negative cross-peak observed between the C^{α} protons of Ala and Pro corresponds to the *cis*-configuration of the Ala-Pro peptide bond in the minor conformer of tetrapeptide *I*, whereas NOEs between the C^{α} proton of Ala and C^{δ} protons of Pro correspond to the *trans*-configuration of this peptide bond in the major conformer.

NOE cross-peaks between neighbouring backbone protons (*d* connections[16]) yield valuable information on the spatial structure of peptides. Intensive NOE cross-peaks of $d_{\alpha N}$ connections were observed for the Lys and Phe residues in both conformers of tetrapeptide *I*. A relatively strong NOE was observed between the Phe and Ala NH protons (Phe d_{NN} connection) in the *trans*-conformer. Similar d_{NN} connections were not observed in the *cis*-conformer and between the Lys and Phe NH protons of the *trans*-conformer. These data suggest the existence of a backbone turn in the

TABLE I

Chemical shifts δ (ppm, 30°C), ^3J(HNC$^{\alpha}$H) values (Hz) and temperature coefficients $\Delta\delta/\Delta T$ ($.10^3$ ppm/K) measured for tetrapeptides *I*–*IV* in DMSO

Tetrapeptide	Lys			Phe			Ala(Thr)		
	δ_{NH}	^3J	$\Delta\delta/\Delta T$	δ_{NH}	^3J	$\Delta\delta/\Delta T$	δ_{NH}	^3J	$\Delta\delta/\Delta T$
Boc-Lys-Phe-Ala-Pro									
trans (57%)	6·70	7·9	6·8	8·49	8·3	8·0	7·62	7·0	1·5
cis (43%)	6·45	7·9	6·5	8·40	8·3	4·7	7·86	6·6	3·8
Boc-Lys-Phe-Thr--Pro	6·60	7·9	12·5	8·58	8·3	8·6	7·34	8·3	2·6
Boc-Lys-Phe-Ala--Ala	6·77	8·0	8·8	8·03	8·8	7·4	7·58	5·0	2·2
Boc-Lys(Z)-Phe--Ala-Pro-ONB	6·87	7·5	10	7·74	8·3	5·0	8·19	7·0	10

vicinity of the Phe residue for the *trans*-conformer of tetrapeptide *I*, which is stabilized by a salt bridge. The low temperature coefficient $\Delta\delta/\Delta T$ measured for the Ala NH proton confirms this suggestion.

Some conclusions can be made by comparing the chemical shifts of amide protons for compounds *I*–*IV* (Table I). Lys NH chemical shifts were roughly equal in all tetrapeptides; on the contrary, the chemical shifts of the other two amide protons were substantially different, especially when comparing tetrapeptide *IV* with blocked ionogenic groups and the *trans*-conformer of tetrapeptide *I* or tetrapeptide *II*. Anomalous chemical shifts probably caused by a specific orientation of anisotropic peptide bonds or intramolecular hydrogen bonding between the amide protons indicate the formation of preferential spatial structure of two latter compounds in solution. It should be noted that chemical shifts of amide protons are more sensitive to conformational changes than the coupling constants $^3J(\mathrm{HNC^{\alpha}H})$ (see Table I).

Comparison of ^{1}H NMR *Data with Results of Conformational Energy Calculations*

The sets of structures obtained by conformational energy minimization for the N-acetyl derivatives of tetrapeptides *I* and *II* are only slightly different. The low-energy conformations of tetrapeptide *I* ($E - E_{\min} < 17{\cdot}0$ kJ mol^{-1}) are given in Table II. It should be noted that the energy of conformers with the *trans*- and *cis*-peptide bond Ala-Pro varies inappreciably. Within the system of potential functions used and at $\varepsilon = 47$ the structures with closely spaced charged groups (the distance between the centres of groups $R_{+-} \lesssim 0{\cdot}5$ nm) and those with sparsely spaced charged groups (R_{+-} up to 1·5 nm) show little energy variation. Experimentally observed differences in the population of *trans*- and *cis*-conformers for tetrapeptides *I* and *II* as well as the presence of a salt bridge in the *trans*-conformers and its absence in the *cis*-conformer of tetrapeptide *I* are apparently due to factors neglected by the semiempirical calculation such as solvent effect and entropy contribution to the peptide free energy in solution.

By comparing ^{1}H NMR spectral parameters (Fig. 4, Table I) with appropriate values predicted for obtained low-energy structures one may attempt to determine the most plausible peptide conformations in solution. Significant restriction of the list of such conformations can be attained using NOE cross-peaks observed in the ROESY spectrum of tetrapeptide *I* (Fig. 4). For instance, intense NOE cross-peaks between the $C_i^{\alpha}H$–$N_{i+1}H$ protons and the absence of NOE between the N_iH–$N_{i+1}H$ protons correspond to L-amino acid conformations with $\psi_i > 60°$ (refs[17,18]). Such limitations can be imposed on the Lys conformation in the *trans*-conformer and on that of the Lys and Phe residues in the *cis*-conformer of tetrapeptide. Two intensive NOE cross-peaks corresponding to the connections $d_{\alpha N}$ and d_{NN} for the Phe residue in the *trans*-conformer are indicative of a low absolute angle value ψ_3

or of an equilibrium between conformations with positive and negative ψ_3 values[18]. Finally, NOE cross-peaks between the $C_i^{\alpha}H$ and N_iH protons are absent in the ROESY spectrum (Fig. 4). NOE cross-peaks of this type can be partially suppressed by the opposite-phase cross-peaks of scalar spin-spin interaction[14]. However, it is hardly plausible that the $C_i^{\alpha}H$–N_iH NOE cross-peak is completely absent, if conformations with $\varphi_i > 0°$ where these protons are closely spaced ($d_{N\alpha} \simeq 0{\cdot}22$ nm) are fairly well represented in solution[17,18].

Consequently, to select structures present in DMSO with considerable weights one can exclude type A conformations of the Lys residue and type A* conformations of any residue for both of the tetrapeptide *I* *trans*-conformers, as well as Phe conformations A and B in the *cis*-conformer (one-letter notation for residue conformations[10]). The *trans*-conformer structures **1** – **5** and *cis*-conformer structures **1** – **6** satisfying these limitations were compared with NMR data (see Table III). Similar values of vicinal constants and temperature coefficients for the amide protons of tetrapeptide *II* and tetrapeptide *I* *trans*-conformer (Table I) suggest similarity of their space structures. Therefore, calculated structure of tetrapeptide *II* with the peptide

TABLE II

Low-energy conformations of Ac-Lys-Phe-Ala-Pro ($\varepsilon = 47$)

No.	*trans*-Ala-Pro			No.	*cis*-Ala-Pro		
	Backbone conformation[a]	$E - E_{min}$ kJ mol^{-1}	R_{+-}[b], nm		Backbone conformation[a]	$E - E_{min}$ kJ mol^{-1}	R_{+-}[b], nm
1	EDD	0·0	0·45	1	EEE	9·2	1·21
2	EDE	13·4	0·49	2	CEE	8·4	1·52
3	CBE	13·4	0·46	3	EEF	14·6	0·91
4	CBF	6·3	0·50	4	CEF	11·3	1·31
5	EAF	9·2	0·34	5	FCF	10·9	0·32
6	AEE	16·3	1·54	6	CFF	14·6	0·37
7	AAD	11·7	0·70	7	EAE	2·1	0·40
8	AA*E	6·7	0·36	8	EAF	3·3	0·65
9	ECA*	5·0	0·53	9	EBD	7·1	0·84
10	AEA*	7·9	1·46	10	AEE	6·3	1·23
11	EAA*	1·7	0·82	11	AAE	15·5	0·87
12	AAA*	10·5	1·30				

[a] One-letter code[10] is used to designate backbone conformations for residues 1–3. Proline, taking into account the symmetry of the terminal COO^- group, has similar backbone conformations in all structures. [b] Distances between the centres of charged groups: lysine ε-amino group nitrogen and C-terminal carboxyl carbon.

TABLE III

Comparison of calculated characteristics for low-energy conformers of the tetrapeptides Ac-Lys-Phe-Ala-Pro and Ac-Lys-Phe-Thr-Pro with ^{1}H NMR spectroscopy data

Tetrapeptide	Conformer No.	Backbone type	^3J(HNC$^\alpha$H)		Accessible amide proton surfaces, nm^2 . 10^2			Interproton distances, nm			
			Phe	Ala(Thr)	Lys	Phe	Ala(Thr)	$d_{\alpha N}(1,2)$	$d_{NN}(1,2)$	$d_{\alpha N}(2,3)$	$d_{NN}(2,3)$
I *trans*	1	EDD	9·9	7·7	4·8	4·1	0·0	0·26	0·44	0·29	0·26
	2	EDE	10·0	6·8	4·5	3·8	0·0	0·24	0·44	0·30	0·26
	3	CBE	9·0	5·4	9·3	4·1	0·0	0·22	0·45	0·28	0·32
	4	CBD	7·4	8·1	9·6	2·2	0·5	0·23	0·41	0·34	0·24
	5	EAF	6·0	5·9	5·0	5·5	1·2	0·24	0·44	0·36	0·24
	experiment[a]		8·3	7·0	6·8	8·0	1·5	+	−	+	+
I *cis*	1	EEE	6·8	8·1	4·4	3·5	2·2	0·25	0·44	0·24	0·44
	2	CEE	6·5	8·1	7·5	0·3	2·2	0·24	0·39	0·24	0·44
	3	EEF	8·1	5·1	4·1	4·1	5·9	0·24	0·44	0·24	0·44
	4	CEF	8·4	5·2	4·3	0·0	6·3	0·24	0·39	0·24	0·44
	5	FCF	3·5	7·0	9·2	3·7	5·1	0·22	0·45	0·29	0·45
	6	CFF	9·5	4·7	8·9	2·8	6·3	0·22	0·44	0·24	0·45
	experiment[a]		8·3	6·6	6·5	4·7	3·8	+	−	+	−
II[b] *trans*	1	EDD	9·9	8·9	4·8	3·1	0·0				
	2	EBE	10·0	9·7	4·4	2·6	0·0				
	3	CAF	6·0	6·4	9·2	1·0	0·0				
	4	CAD	6·0	9·5	9·6	1·3	0·2				
	5	EAF	6·2	6·4	4·9	5·4	0·0				
	experiment[a]		8·3	8·3	12·5	8·6	2·6				

[a] Accessible amide proton surfaces in calculated conformers are compared with the temperature coefficients $\Delta\delta/\Delta T$ (.10^3 ppm/K), interproton distances with the absence (−) or presence (+) of NOE cross-peaks in ROESY spectrum. [b] ROESY spectrum of tetrapeptide *II* was not measured and interproton distances were not calculated.

bond Thr-Pro in the *trans*-configuration satisfying the restrictions outlined above were selected for comparison with experimental data (Table III).

Most vicinal constants (^3J(HNC$^\alpha$H)) calculated by means of the Karplus–Bystrov equation[19] for Phe and Ala residues in structures with a *trans*-peptide Ala-Pro bond do not deviate from the observed values by more than 2 Hz. The ^3J(HNC$^\alpha$H) values for the first residue are not included in comparison since the effect of the bulky Boc group is not taken into account during calculations. It should be pointed out that calculated ^3J(HNC$^\alpha$H) values are distributed on both sides of experimental values, i.e. more close conformity can be achieved by assuming an equilibrium of several conformers. Conformation 5 appears to be the only one predicting a much underestimated vicinal constant value for the Phe residue.

Temperature coefficients $\Delta\delta/\Delta T$ characterize the accessibility of amide protons to solvent. For calculated structures similar characteristics are provided by accessible surface areas of amide protons[20,21] estimated with the aid of an algorithm[22]. In structures with the *trans*-peptide bond Ala-Pro a clearcut correlation is observed between the calculated and experimentally found values of amide proton accessibility (Table III), viz. the Ala NH is shielded from solvent, while the other two amide protons are exposed. Calculated proton–proton distances, all except for the connection $d_{\alpha N}(2, 3)$ in the *trans*-conformers 4 and 5, agree qualitatively with the experimental NOE set of tetrapeptide *I*.

In all *trans*-conformers of tetrapeptide *I* presented in Table III, the Lys ε-amino group and the C-terminal carboxyl group are $R_{+-} < 0{\cdot}5$ nm apart. Conformations 1–3 are also consistent with other NMR data. A combined stereoview of these conformers depicted in Fig. 5 demonstrates that they all represent somewhat different

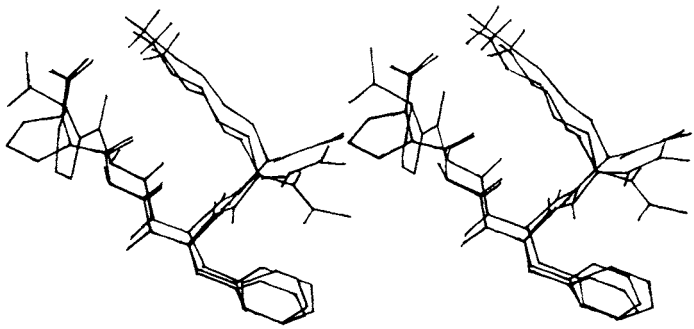

FIG. 5

Superimposed stereoviews of tetrapeptide *I* in conformations 1–3 with a *trans*-peptide bond Ala-Pro (see Tables II and IV)

variants of a common type of spatial organization of the tetrapeptide *I* molecule. The structure is characterized by lysine side chain densely packed in parallel with the Ala-Pro dipeptide backbone. The salt bridge is stabilized not only by the electrostatic attraction and hydrogen bonding involving ionized groups, but also by the Van-der-Waals contacts between the two parallel regions in the molecule. An additional stabilizing factor can be provided by interaction between the Phe ring and the N-terminal Boc group not included in the calculations. The amide proton of Ala in the proposed model fails to participate in intramolecular hydrogen bonding and is completely shielded from solvent by the adjacent backbone regions and the lysine side chain.

Of the calculated tetrapeptide *II* conformations given in Table III conformers 1 and 2 are the only ones showing good agreement with the available NMR data. Incidently, they predict a certain rise in the value of the Thr $^3J(\mathrm{HNC^{\alpha}H})$ vicinal constant relative to the *trans*-conformer of tetrapeptide *I*. Both these conformers closely resemble the space structure model proposed for the *trans*-conformers of tetrapeptide *I* (Fig. 5). The remaining conformers of tetrapeptide *II* suggest a low value for one of the vicinal constants and/or partial shielding of the Phe amide proton by weak intramolecular hydrogen bonding, which is at variance with the temperature dependence of the chemical shift of this proton.

Among conformers with the *cis*-peptide Ala-Pro bond satisfying the experimental set of NOE only two of them (5 and 6) permit the formation of a salt bridge. These conformers predict a significantly underestimated value of one of the vicinal constants $^3J(\mathrm{HNC^{\alpha}H})$. The *cis*-conformer of tetrapeptide *I* is characterized by almost the same availability of the three amide protons to solvent. In comparison with the *trans*-conformer the temperature coefficient $\Delta\delta/\Delta T$ is diminished in the Phe NH but is increased in the Ala NH. In the calculated *cis*-conformers of tetrapeptide *I* the Ala NH is more exposed to solvent than in the *trans*-conformers, whereas the Phe NH in some *cis*-conformers is shielded from solvent.

^{1}H NMR data available for the tetrapeptide *I* *cis*-conformer are consistent with the suggestion that a statistical equilibrium exists in solution between several structures shown in Table III. All amino acid residues of the *cis*-conformers in Table III belong to the common low-energy region ($\phi < 0°$, $\psi > 60°$) of Ramachandran's conformational map. Energy barrier between individual local minima within this region are small and easy to overcome. A transition to the *cis*-configuration of the Ala-Pro peptide bond results in a folded conformation of the C-terminal region of the backbone, the carboxyl group being removed from the side chain of lysine. It has been estimated that in this case, too, a closely spaced location of charged groups is sterically feasible. However, whereas the lysine ε-amino group in the *trans*-conformer readily "finds" the carboxyl group by "moving" along the extended C-terminal part of the backbone, a random contact of ionized groups in the *cis*-conformer in the absence of additional stabilizing interactions fails to materialize in a stable

salt bridge (cf. the superimposed stereoviews of two *cis*-conformers of tetrapeptide *I* in Fig. 5).

The aforesaid is indirectly supported by NMR spectroscopy data for the Boc-Lys--Phe-Ala-Ala tetrapeptide (Table I). The low value of the vicinal constant $^3J(HNC^{\alpha}H)$ for Ala[3] indicates a folded conformation for the C-terminal region of this tetrapeptide, whereas equivalence of chemical shifts of the Lys $C^{\varepsilon}H_2$ protons suggests a lack of a stable ionic bond.

Table IV gives dihedral angle values for the calculated *trans*-conformers of tetrapeptides *I* and *II* compatible with the NMR data. The common type of spatial arrangement shared by these conformers (Fig. 5) resembles a β-turn, whose N--terminal arm is formed not by the peptide backbone but by the extended aliphatic

TABLE IV

trans-Conformers of the tetrapeptides Ac-Lys-Phe-Ala-Pro and Ac-Lys-Phe-Thr-Pro consistent with NMR spectroscopy data

Tetrapeptide	Backbone type	Dihedral angles								
		Lys					Phe		Ala(Thr)	
		χ_1	χ_2	χ_3	χ_4	ψ	ϕ	ψ	ϕ	ψ
I	EDD	61	164	180	168	172	−125	35	−151	77
	EDE	61	178	−178	173	162	−118	31	−156	155
	CBE	−165	−167	−179	−173	117	−101	46	−165	156
II	EDD	61	164	−179	169	173	−125	26	−140	78
	EBE	61	176	179	173	165	−119	15	−130	157

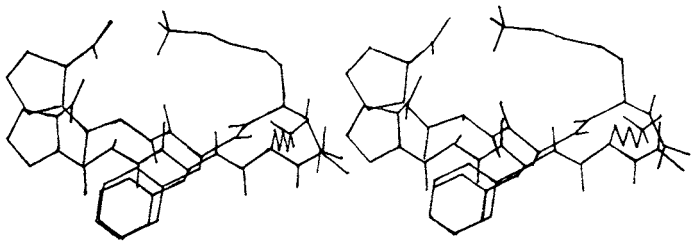

FIG. 6

Superimposed stereoviews of tetrapeptide *I* in conformations 1 and 5 with a *cis*-peptide bond Ala-Pro (see Table II)

part of the lysine side chain. It can be referred as the Lys-turn in line with the Asx-turns proposed recently[24]. The conformation of one of these corner residues (Phe) in this unusual turn is similar to conformations of the i + 2 residue in the conventional type I and II' β-turns[23]. Another corner within this turn formed by the backbone and Lys side chains can be typified by the unconventional torsional angles C^{γ}-C^{β}-C^{α}-C' ($\phi' = \chi_1 - 120°$) and C^{β}-C^{α}-C'-N' ($\psi' = \psi - 240°$). Using these notations the similarity shared by the Lys-turn and the standard β-turns becomes apparent: two distinct lysine conformations in Table IV ($\phi' \simeq -60°$, $\psi' \simeq -70°$ and $\phi' \simeq 75°, \psi' \simeq -120°$) closely resemble conformations of residues i + 1 in the type I and II' β-turns[23]. Such side chain-backbone turns may represent a preferable spatial arrangement of the long side chains, which leads to intramolecular salt bridges or to optimization of hydrophobic contacts with the environment.

CONCLUSION

As follows from this study, the space structure of tetrapeptides *I* and *II* is characterized by intramolecular interaction between the lysine ε-amino group and the C-terminal carboxyl of the backbone. This interaction is reflected in the ^{1}H NMR spectra by nonequivalence of $C^{\varepsilon}H_2$ protons in the side chain of Lys. Difference in the chemical shifts of these protons in tetrapeptide *I* (*trans*-conformer) and *II* amounts to $\Delta\delta = 0{\cdot}19$ ppm, while tetrapeptide *III* only shows a broadened multiplet instead of a triplet. Thus, the extent of Lys $C^{\varepsilon}H_2$ proton unequivalence may serve as a qualitative measure of salt bridge stability.

A comparison of NMR data obtained for the tetrapeptide series under study with results of conformational energy calculations suggests that interaction of charged groups in the DMSO environment is not the major factor responsible for space structure stabilization. A stable salt bridge is only found in molecules possessing certain conformational rigidity, whereas an increase in backbone mobility after transition of the peptide X-Pro bond to *cis*-configuration or following the substitution of proline for alanine leads to ionic bond breaking. Hence, a gain in free energy when ionic groups become closely arranged in DMSO does not appear large enough to compensate for the entropy changes caused by fixation of the space structure of a conformationally labile peptide.

The functional role of salt bridges during the interaction of oligopeptides with lipids and proteins remains an open question. Further physico-chemical investigations along these lines by using model compounds with stable salt bridges (e.g., tetrapeptide *II*) appear worthwhile.

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