The features of the spatial structure of the gramicidine A–cesium complex

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Abstract Earlier obtained two-dimensional ^{1}H-NMR spectroscopy data were used to analyze the spatial structure and conformational mobility of the double right ↑↓ $\pi\pi^{7,2}_{LD}$ helix of the complex formed by gramicidine A and Cs^+ ions in an organic solvent (a chloroform–methanol mixture). Analysis of the experimental data permitted the determination of a set of conformations for each of the high-mobility residue side chains in the solution. The energy refinement of the most probable conformation of the double right ↑↓ $\pi\pi^{7,2}_{LD}$ helix was made and conformational rearrangements of the tryptophan residue side chain were studied in detail.

Key words: Gramicidine A; Ion channel; ^{1}H NMR; Conformational mobility

1. Introduction

Pentadecapeptide gramicidine A (GA) is a natural antibiotic. Its dimers form ion channels in bilayer membranes [1,2]. As shown in [3], GA-analogues form long-living channels in which their spatial organization (supposedly a double helix) basically differs from the head-to-head conformation. Under certain conditions, GA itself forms these channels [4]. Therefore, interest in alternative conformations of the GA membrane channel is quite natural. The mutual orientation of the GA molecules forming a dimer has been determined in [5] from NMR data. However, the spatial structure GA–Cs^+ complex (CSGA) could not be determined by usual methods [6,7], because the majority of the residue side chains had high conformational mobility. The main aim of this work was to detect a full set of residue side chain rotamers by multistep analysis of the available experimental data. The methodology used in the present work can be successfully applied to determine the spatial structures of other high-mobility peptides.

2. Experimental

The populations of side chain rotamers were calculated as described in [8] from experimental H–C^α–C^β–H spin–spin coupling (SSC) constants. When constructing a correlation diagram $^3J_{\alpha\beta1}(\chi^1)$–$^3J_{\alpha\beta2}(\chi^1)$ (Fig. 1), SSC constants were calculated by [9], where the ratio of the dihedral angle θ to the torsion angle χ^1 was determined according to the IUPAC-IUB Nomenclature [10]. The influence of dynamic averaging for the SSC constant values was approximately by a Gaussian distribution function for χ^1.

A computer visualization of the ↑↓ $\pi\pi^{7,2}_{LD}$ helix model proposed in [5] was used to analyze NOE contacts for various possible side chain conformations. The side chain conformations of residues were analyzed for the *t*-, g^+- or g^--rotamers of torsion angles. The energy refinement of the ↑↓ $\pi\pi^{7,2}_{LD}$ helix was performed by the CONFORNMR program [11] using nonvalent interaction potentials [12]. To analyze the mobility of L-Trp side chains, the GA right ↑↓ $\pi\pi^{7,2}_{LD}$ helix was calculated (all α-substituted residues except the L-Trp residue being investigated were replaced with alanine residues). In this way L-Trp surrounded by amino acid residues with highly mobile side chains and without steric obstacles to the L-Trp side chain movement was modeled. Conformations with potential energy less than that of the ↑↓ $\pi\pi^{7,2}_{LD}$ helix built from the alanine residues, i.e. –261 kcal/mol, appeared to be sterically resolved.

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3. Results and discussion

The obtained set of NOE cross-peaks made it possible to establish that the gramicidine A–cesium (CsGA) complex is a double right ↑↓ $\pi\pi^{7,2}_{LD}$ helix [5]. Most of its side chains are apparently very mobile, which was confirmed by averaged H–C^α–C^β–H coupling constants ranging from 6 Hz to 8 Hz (Fig. 1) as well as by the set of NOE cross-peaks, which cannot be associated with a single conformation.

The calculation of χ^1-rotamer populations showed that the populations of the L-Val[7] t-rotamer, L-Trp[11,13] g^--rotamer and D-Leu[14] g^+-rotamer in CsGA are low (<10%), and the populations of the L-Trp[11] g^+-rotamer, D-Leu[14] *t*-rotamer, and L-Trp[15] *t*-rotamer are predominant (>60%); for the rest of the residues, the populations of χ^1-rotamers are evidently close.

The two-dimensional NOESY spectrum of the CsGA complex shows 198 NOE cross-peaks involving side chain protons. They can be attributed to 337 different proton pairs. Some ambiguities might be eliminated, if protons pairs known to be distant (e.g. those situated on opposite ends or sides of the double helix) are not considered. Thus, 136 unambiguously interpreted NOE cross-peaks were isolated.

After the set of unambiguously interpreted NOE cross-peaks had been obtained, analysis of possible conformations of each side chain was made using the model of ↑↓ $\pi\pi^{7,2}_{LD}$ helix. The possibility of NOE contact between the protons corresponding to the unambiguously interpreted NOE cross-peaks was estimated for each conformation.

3.1. Conformations of Val Residues

The spatial contiguity of Val[1,7,8] protons corresponding to the unambiguously interpreted NOE cross-peaks was estimated for two versions of stereochemical assignment of signals of $C^\gamma H_3$-groups. According to the analysis, the observed NOE cross-peaks can be explained only if we assume that the residues Val[1,7,8] have two or more χ^1-rotamers. This agrees well with the above estimation of rotamer populations (Table 1).

For the residue L-Val[1] the NOE cross-peak system can be explained by the combination of *t*-, g^--rotamers for one version of stereochemical assignment of H^γ-protons or by the combination of *t*- and g^+-rotamers for the other; this agrees with the experimental H–C^α–C^β–H coupling constant. Chemical shifts of the $C^{\gamma 1}H_3$- and $C^{\gamma 2}H_3$-groups of D-Val[6] coincide, so the analysis of NOE cross-peaks does not determine χ^1-rotamers.

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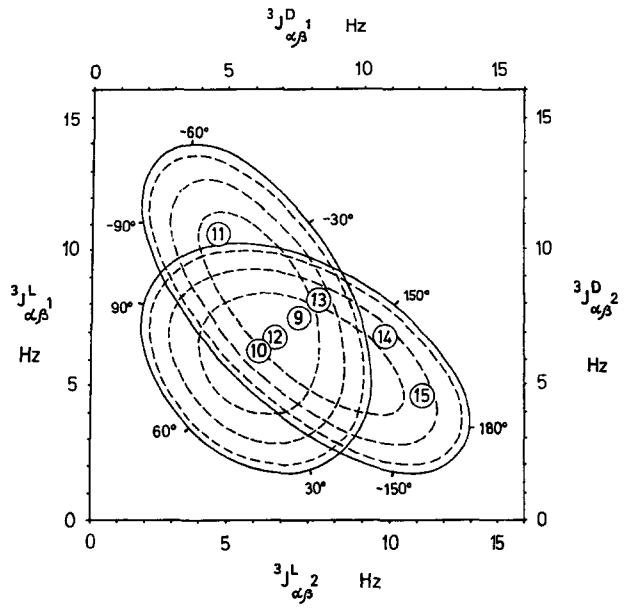

Fig. 1. Correlation diagram of coupling constant $^3J_{\alpha\beta1}$–$^3J_{\alpha\beta2}$. The solid line shows the relationship between SSC constants (fixed values of the torsion angle χ^1). The dashed line shows the same relationship for side chain rapidly rotating arround the χ^1-angle, charcterized by Gaussian distribution with dispersion values of σ = 10°, 20° and 30°. Experimental values of spin–spin coupling constants for L-Trp[11,15] and D-Leu[14] have been arranged in accordance with the most probably conformation (Fig. 2).

DVal[6] H–C^α–C^β–H SSC constant allows for the determination of the total population of t-, g^+-rotamers (75%). NOE contacts of L-Val[7] can be explained by several existing rotamer pairs. However, due to the low population of the t-rotamer (5%) the rotamer pair of g^+g^- (Table 1) is preferable. In the residue D-Val[8] the total population of the t- and g^+-rotamers is 70%, which explains why we cannot choose between the two rotamers pairs (t, g^+ and t, g^-). Analysis showed that the NOE data and H–C^α–C^β–H coupling constants quite agree with each other; in order to explain the experimental data it is sufficient to assume that only two rotamers are present for each residue. Nevertheless, all three rotamers might exist in the residues Val[1,6,8].

3.2. Conformations of Leucine Residues

In D-Leu[4] chemical shifts of H^β-protons coincide (Table 1 [5]), but unambiguously interpreted NOE contacts involving protons of C^γH-, $C^{\delta1}$H- and $C^{\delta2}$H-groups are not observed. Therefore NMR spectroscopic data do not allow us to form a conclusion regarding side chain conformation. The NOE cross-peaks for D-Leu[10] can be explained by the presence of t- and g^+-rotamers, whereas estimation of rotamer populations based on the SSC constant values (Table 1) indicates that the g^--rotamer is realized as well. A similar situation is observed for D-Leu[12] where Noe contacts can be explained by the presence of the t-rotamer, and the experimental values of coupling constants point to quantitatively similar populations of the three χ^1 rotamers (Table 1). No NOE data are available on the residue D-Leu[14], and t- and g^--rotamers appear to be the most populated (Table 1).

3.3. Conformations of triptophan residues

Bulky and asymmetric side chains of tryptophan residues essentially facilitate analysis of their conformations with the use of the $\uparrow\downarrow \pi\pi^{7,2}_{LD}$ helix model. The presence of a large number of NOE cross-peaks involving spectrally distinguishable protons of indole rings allows for thorough investigation of side chain mobility. Assuming that two or more conformational states of tryptophan side chain are simultaneously present in a solution one can explain the NOE cross-peak system for tryptophan residues. The peculiarity of the combinations of alternative rotamer pairs is due to a difference in only one of the torsion angle χ^1 or χ^2 (Tables 1 and 2). For the residues L-Trp[9,13] H–C^α–C^β–H coupling constants indicate that two χ^1-rotamers (t and g^+) are characterized by greatest populations. However, the L-Trp[13] 'minor' rotamer population (g^-) is much lower. For L-Trp[9] the NOE cross-peaks can be explained by two possible pairs of conformations – tg^+, g^+g^+ and tg^-, g^-g^-, and for L-Trp[13] they can be explained only by the pair tg^+, g^+g^+ (Tables 1 and 2). The H–C^α–C^β–H coupling constant indicates that the residues L-Trp[11,15] have a χ^1 rotamer (t or g^+) with predominant population (>60%) and two 'minor' rotamers with populations of 20% and 10% (t and g^- for L-Trp[11] and g^- or g^+ for L-Trp[15]). Any of three different pairs of rotamers can be used to explicate the NMR data (Table 2). Essential information can be obtained by additional study of NOE cross-peaks between the protons of the $C^\beta H_2$-group of L-Trp[11,15] and the amide protons of its own (the i-th) and the next (the i+1-th) peptide groups.

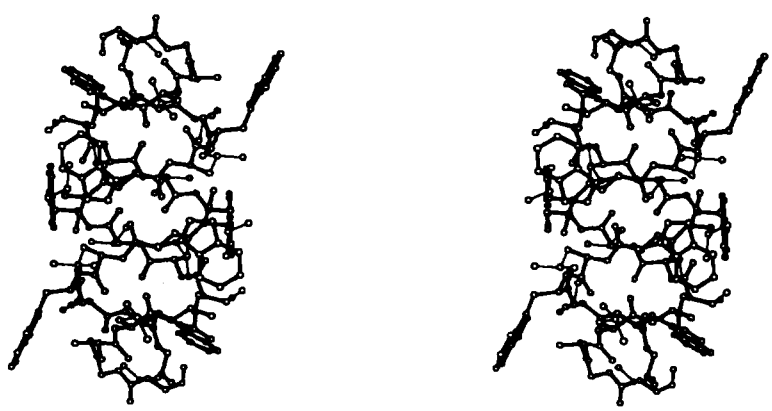

Fig. 2. The most probable conformation of right $\uparrow\downarrow \pi\pi^{7,2}_{LD}$ helix (a stereoview).

In the case of L-Trp[11,15] residues intensive NOE cross-peaks with similar amplitude assigned to H^{β}_{i}-protons and Nh_i-proton were observed. One of the H^{β}_{i}-NH_{i+1} cross-peaks of L-Trp[11] residue was less intensive, and both H^{β}_{i}-NH_{i+1} cross-peaks of L-Trp[15] were absent. This, with known side chain conformation, makes it possible to select torsion angles χ^1 for the residues: $\chi^1 \sim 180°$ for L-Trp[15] and $\chi^1 \sim 60°$ for L-Trp[11]. Therefore, it is quite sufficient to suppose that L-Trp[15] features the rotamer pair tg^+, tg^-, and L-Trp[11] features the rotamer pair g^+g^-, g^+g^+, maybe with a small touch of 'minor' rotamers.

Since only one signal corresponds to each proton in the ^{1}H-NMR spectra, residue chain conformations (Table 2) must rapidly (on the NMR time scale) convert into each other. These transitions seem to be most difficult for the bulky side chains of tryptophan residues. Therefore, it is of interest to analyze steric conditions of these conformational transitions for tryptophan residues placed on the central and terminal positions of the right $\uparrow\downarrow$ $\pi\pi^{7,2}_{LD}$ helix (in positions 9 and 15, respectively). Calculations demonstrated that there are six interrelated, sterically allowable regions in χ^1–χ^2 dimensions: g^-g^+, tg^+, g^+g^+, g^-g^-, tg^-, g^+g^-. Analysis of the sterically allowed regions for Trp residue in position 9 showed the existence of the following transition routes in χ^1–χ^2 space: tg^+<--->g^+g^+, tg^+<--->g^+g^-, tg^-<--->g^+g^-, tg^-<--->g^-g^-, g^+g^+<--->g^-g^+, g^+g^+<--->g^+g^-, g^+g^-<--->g^-g^-, g^-g^-<--->g^-g^+. It is seen from Tables 1 and 2 that the conformational mobility of the side chain of most Trp residues can be realized by transitions between adjacent regions in the χ^1-χ^2 space that are sterically allowed and differ from each other only by the value of either χ^1 or χ^2. At first glance, the Trp[15] residue seemed to be an exception owing to its mobility in the most probable conformation (tg^+; tg^-) passing the adjacent sterically allowed regions in the order tg^+<--->g^+g^+<--->g^+g^-<--->tg^- or tg^+<--->g^-g^+<--->g^-g^-<--->tg^-. However, estimation of the rotamer population (Table 1) is inconsistent with the assumed scheme of transitions. Indeed, closed examination of sterically allowable regions demonstrated that the direct transition tg^+<--->tg^- is possible for Trp[15], because this residue is in terminal position.

To illustrate the spatial stucture of the $\uparrow\downarrow$ $\pi\pi^{7,2}_{LD}$ helix, the energy refinement of a dimer conformation with one of the most probable combinations of side chain rotamers was used.

Table 1
The side chain rotamers of CSGA residues
Analysis of H–C^{α}–C^{β}–H coupling constants and NOE contacts

Residue	N[a] NOE	p[b] (%)			Rotamers set by NOE data[c] (χ^1, χ^2)	
		t	g^+	g^-		
L-Val[1]	17	38		62	***t*** (14)	g^- (10)
D-Leu[4]	0					
D-Val[6]	0		75	25		
L-Val[7]	17	5		95	$\boldsymbol{g^+}$ (15)	g^- (7)
					t (12)	g^- (7)
D-Val[8]	8		70	30	***t*** (4)	g^- (4)
L-Trp[9]	8	38	38	24	$\boldsymbol{t\,g^+}$ (6)	g^+g^+ (2)
					$t\,g^-$ (3)	g^+g^- (5)
D-Leu[10]	10	27	46	27	***t*** - (9)	g^+ – (5)
L-Trp[11]	12	23	63	10	$\boldsymbol{g^+\,g^+}$ (7)	g^+g^- (9)
					$t\,g^-$ (5)	g^+g^- (9)
					$t\,g^+$ (5)	g^+g^+ (7)
D-Leu[12]	3	32	46	32	***t*** – (3)	
L-Trp[13]	5	46	46	8	$\boldsymbol{t\,g^+}$ (4)	$t\,g^-$ (3)
D-Leu[14]	0	60	10	30		
L-Trp[15]	15	75	8	17	$\boldsymbol{t\,g^+}$ (10)	$t\,g^-$ (6)
					g^+g^- (11)	g^+g^+ (3)
					g^+g^- (11)	g^-g^- (4)

[a] The number of unambiguously interpretable NOE cross-peaks from protons of $C^{\gamma}H_3$-, $C^{\delta}H_3$-groups, indole ring protons, and backbone protons contacted with the side chain protons of neighbouring residues.
[b] Populations of the χ^1-rotamers. Underlined are rotamers which cannot be differentiated by H-C^{α}-C^{β}-H spin-spin coupling constants.
t, corresponding value of torsion angle ~ 180°
g^+, ~ –60° (for χ^2 L-Trp ~ –60° : –120°)
g^-, ~ +60° (for χ^2 L-Trp ~ +60° : +120°)
[c] In parentheses is the number of unambiguously interpretable NOE cross-peaks which can be explained by side chain conformations. The rotamer combinations typed in bold were used for the most probable structure (Fig. 2).

Table 2
Unambiguously interpreted NOE cross-peaks of tryptophane indole rings and interproton contacts for different rotamers

NOE contact		Rotamer (χ^1, χ^2)					
		tg^+	tg^-	g^+g^+	g^+g^-	g^-g^+	g^-g^-
Trp[9]							
N1H (10.39)	H^{γ} Val[7] (1.02) ic		*	*			
C2H (7.20)	H^{α} Trp[9] (5.33)		*	*			
	HN Leu[10] (8.87)	*	*				*
	HN Trp[9] (9.04)	+			*	*	
C4H (7.77)	H^{γ} Val[7] (1.02) ic	*			*		
	H^{γ} Val[7] (1.16) ic	*			*		
	H^{α} Trp[9] (9.04)	*			*		
C7H (7.46)	H^{γ} Val[7] (1.02) ic	*			*		
Trp[11]							
N1H (10.65)	H^{δ} Leu[10] (0.24)			*	*		
	H^{β} Ala[5] (1.54) ic		*	*	+		
C2H (7.11)	H^{β} Ala[5] (1.54) ic		*	*			
	H^{α} Trp[11] (5.08)		*	*			
	HN Ala[5] (9.30) ic		*	*			
	HN Trp[11] (9.34)	+			*	*	
C4H (7.78)	H^{α} Trp[11] (5.08)	*			*		
	H^{β} Ala[5] (1.54) ic	*			*		
	HN Leu[12] (9.09)	+	+		*	*	
C5H (7.19)	H^{β} Ala[5] (1.54) ic	*			*		
C6H (7.31)	H^{δ} Leu[10] (0.24)			*	*		+
C7H (7.56)	H^{δ} Leu[10] (0.24)			*	*		+
Trp[13]							
N1H (10.52)	H^{γ} Val[1] (0.87) ic	*	*				
	H^{γ} Val[1] (0.98) ic	*	*				
C2H (7.16)	H^{β} Ala[3] (1.48) ic		*	*			
	H^{α} Leu[14] (4.93)	*					*
C6H (7.26)	H^{δ} Leu[12] (1.27)	+		*			*
Trp[15]							
N1H (10.32)	H^{γ} Val[1] (0.98) ic		*	*	+		
	H^{γ} Val[7] (1.16)	*				*	*
C2H (7.06)	H^{γ} Val[1] (0.98) ic		*	*			
	H^{γ} Val[7] (1.16)	*			+	*	*
	H^{α} Trp[15] (5.21)		*	*		+	+
	HN Val[1] (8.27) ic	*			*		
	HN Trp[15] (8.57)	+			*	*	
C5H (7.09)	H^{γ} Val[1] (0.87) ic	*			*		
	HN Val[1] (8.27) ic	*			+		
C4H (7.62)	H^{γ} Val[1] (0.98) ic	*			*		
	H^{γ} Val[7] (1.16)		*			*	+
	H^{α} Val[1] (4.87) ic	*			*		
	H^{α} Trp[15] (5.21)		*		*		
	HN EA (8.52)	+	+		*	*	
C6H (7.26)	H^{γ} Val[1] (0.87) ic	*			*		

The rotamers which yield only low intensity NOW cross-peak are indicated by '+' sign. The symbol 'ic' designates interchain NOE contacts. Proton chemical shift is noted in parentheses.

The resulting structure (Fig. 2) completely lacked steric stresses, and lengths of interchain hydrogen bonds are close to 'perfect' and fall in the range between 1.79 and 2.03 Å.

The analysis has shown that using the ^{1}H-NMR spectroscopy data it is possible to determine the conformation set that characterizes the spatial structure of mobile polypeptides.

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