Equilibrium of conformers in solution: spin-labelled angiotensin

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The number of structural parameters were determined for spin-labelled angiotensin in aqueous solution with the use of fluorescence spectroscopy and ^{1}H NMR relaxation induced by the spin label. At the same time all measured parameters were estimated theoretically by means of energy calculations and Monte-Carlo techniques. The matching procedure for experimental and computational data allows one to suggest a dynamic equilibrium between conformers of the molecule in aqueous solution and to estimate the values of their weights.

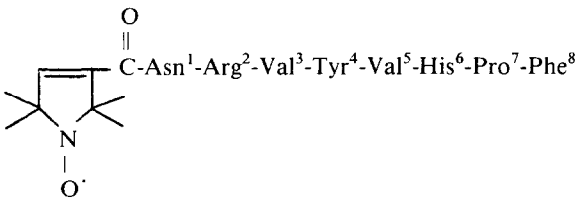
Energy calculation *Angiotensin* *NMR relaxation* *Peptide solution conformation*

1. INTRODUCTION

The procedure of weight selection for low-energy conformers aimed at ensuring the closest fit with the data of physico-chemical experiments in solution has been discussed in our previous work describing CD spectra of cyclotuftsin [1]. The same approach has been applied here to a much more complicated case, namely the molecule of spin-labelled angiotensin (SL-AT). The structure of the molecule in aqueous solution was investigated by fluorescence spectroscopy and ^{1}H NMR relaxation induced by the spin label.

2. METHODS AND RESULTS

SL-AT was prepared by attaching a nitroxide radical to the N-terminal amino group of the AT peptide:



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The values of spin-lattice relaxation times T_{1M} for several protons of the molecule were determined with the pulse sequence $180°-\tau-90°-T_\infty$ using a Bruker WM-360 instrument. It is noteworthy that in the case of relatively large peptides, such as AT, determination of spin-spin relaxation time by linewidth measurements is usually unsuccessful due to signal overlapping. For ^{1}H NMR investigation, the peptide concentration was less than 1 mM to prevent intermolecular interaction; pH ranged from 2.2 to 11.3. The values of vicinal coupling constants J(HNC$^\alpha$H) were measured for the molecule when SL had been reduced by ascorbate. The efficiency of singlet-singlet energy transfer (T) between the Phe and Tyr chromophores was measured using a Hitachi-850 spectrofluorimeter (same pH range, peptide concentration $\approx 40\ \mu$M). As a result of these investigations the mean values were estimated for 17 structural characteristics of SL-AT in aqueous solution (see table 1).

On the other hand, energy calculations performed in the same manner as in [1] suggest the existence of 12 types of sterically allowed ($\Delta U \leqslant$ 10 kcal/mol) peptide backbone structures for SL-AT in aqueous solution: (1) BBLRRBBB, 0.7; (2) BRBRRBBB, 4.7; (3) BBRRRBBB, 0.0; (4) BBLRRBRB, 0.1; (5) BRBRRBRB, 9.9; (6)

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Table 1

Calculated and measured characteristics of SL-AT in aqueous solution (pH 8.2)

Parameter	Experiment	Calculated		
		1	2	3
SL-C$^{\alpha}$H Val^3, Å	9.8 ± 0.2	9.6 ± 0.1	0.2	11.2
SL-C$^{\delta}$H Tyr^4, Å[a]	9.7 ± 0.2	9.7 ± 0.2	0.3	8.3
SL-C$^{\epsilon}$H Tyr^4, Å[a]	9.4 ± 0.1	9.7 ± 0.2	0.1	9.1
SL-C$^{\alpha}$H Val^5, Å	11.3 ± 0.2	11.3 ± 0.2	0.2	9.4
SL-C$^{\delta}$H His^6, Å	11.4 ± 0.4	10.2 ± 0.7	0.3	7.7
SL-C$^{\epsilon}$H His^6, Å	10.8 ± 0.2	10.2 ± 0.4	0.3	10.0
SL-C$^{\beta}$H Pro^7, Å[b]	11.5 ± 0.2	11.3 ± 0.2	0.2	13.9
SL-C$^{\delta}$H Pro^7, Å	12.3 ± 0.3	12.9 ± 0.1	0.1	15.7
SL-C$^{\delta}$H Phe^8, Å[a]	11.4 ± 0.2	11.4 ± 0.1	0.1	13.4
r_{48}, Å[c]	12.1 ± 0.2	11.9 ± 0.1	0.3	11.1
J Asn^1, Hz	7.3 ± 0.5	6.4 ± 0.3	0.2	8.7
J Arg^2, Hz	7.1 ± 0.3	7.9 ± 0.4	0.4	7.6
J Val^3, Hz	8.0 ± 0.3	7.6 ± 0.3	0.2	7.5
J Tyr^4, Hz	7.6 ± 0.3	6.9 ± 0.3	0.2	6.3
J Val^5, Hz	8.2 ± 0.3	8.1 ± 0.3	0.2	9.1
J His^6, Hz	7.0 ± 0.3	7.4 ± 0.3	0.0	9.1
J Phe^8, Hz	7.5 ± 0.3	8.4 ± 0.4	0.1	9.9

[a] T_{1M} values were calculated for 2 equivalent protons according to the fast exchange limit: $1/T_1 = 0.5/T_1^a + 0.5/T_1^b$

[b] Corresponding values for C$^{\beta 1}$H and C$^{\delta 2}$H protons were used for T_{1M}^{calc} since the more exact assignment of Pro^7 signals in ^{1}H NMR spectra of AT was unknown

[c] Critical Förster distance for Phe–Tyr, R_0 = 12.1 Å

Columns: 1, results of averaging over the 'minimal' set of structures from fig.1; 2, maximal differences between the means averaged over different non-minimal sets; 3, results of averaging using Boltzmann weights for low-energy structures obtained by energy minimization

BBBRRBBB, 3.8; (7) BBBRRBRB, 4.6; (8) BBRRBBBB, 9.2; (9) RBLRRBBB, 5.6; (10) RRBRRBBB, 4.1; (11) RBLRRBRB, 0.1; (12) RBRRBBBB, 7.8. The symbols B, R and L represent the following combinations of signs for the dihedral backbone angles ϕ and ψ, respectively: −,+; −,−; +,+; accompanying values are the relative conformational energies in kcal/mol obtained by the conventional minimization procedure. Statistical samples of SL-AT structures each containing 15000 conformations were generated by means of Monte-Carlo techniques [2] for each listed region of local energy minima. The mean values and their deviations were calculated within each statistical sample for all values being measured (T_{1M}, T and J values) using the Solomon-Blombergen dependence [3] (with spin-label correlation time value instead of the dipole-dipole relaxation time), Förster equation [4] and Karplus-Bystrov relationship [5].

The selection of conformer weights w_i was performed by minimization of the following object function:

$$\sum_{k=1}^{17} \left\{ \left[A_k^{exp} - \sum_{i=1}^{12} w_i A_{ki}^{calc} \right]^2 \Big/ \left[\left(D_k^{exp} \right)^2 + \sum_{i=1}^{12} \left(w_i D_{ki}^{calc} \right)^2 \right] \right\}$$

which is a combination of Student's coefficients for the Gaussian distributions of the means (A denotes the mean value of any structural characteristic and D its deviation). The $w_i \geqslant 0$ and $\Sigma_{i=1}^{12} w_i = 1$ limitations were observed during the minimization procedure; moreover, $|A_k^{exp} - \Sigma_{i=1}^{12} w_i A_{ki}^{calc}|$ values were chosen so as not to exceed the corresponding 95% confidence limits for any k value.

The object function was shown to possess a single minimum. A w_i set corresponding to the minimal number of conformers and satisfying all the aforesaid limitations was determined within it. The set consists of 6 nonzero weights corresponding to particular SL-AT structures. Further attempts to exclude any structure (especially the 3rd and 8th conformations) lead to a pronounced discrepancy with the experimental data. Table 1 presents a fairly good agreement between measured and calculated intramolecular distances and vicinal coupling constants. The 'indispensable' structures included in the 'minimal' set and their weights are shown in fig.1; both folded and extended conformations are represented among them. Table 1 also reveals a close similarity between the structural characteristics calculated for those 'non-minimal' w_i sets which meet all the above requirements but contain a greater number of structures (excluding the 2nd, 4th, 5th and 10th ones). Moreover, it contains computational data which are obtainable by using Boltzmann weights calculated on the basis of the relative energies for

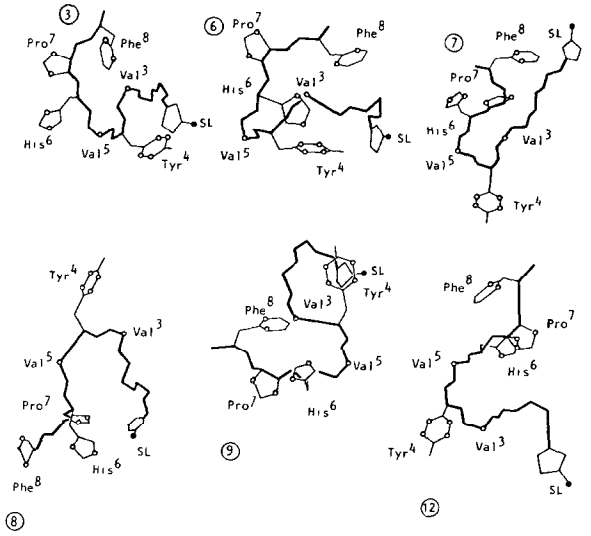

Fig.1. SL-AT structures constituting minimal set (w_3 = 0.26; w_6 = 0.08; w_7 = 0.05; w_8 = 0.07; w_9 = 0.32; w_{12} = 0.22). The empty circles indicate the protons, whose relaxation is affected by the unpaired electron of the SL depicted as a solid circle.

12 low-energy SL-AT structures provided by energy minimization (see above). Table 1 demonstrates a rather poor agreement between the experimental and calculated data in this case; it is interesting to note that this averaging procedure is still being routinely used in the literature.

A change in protonation of the C-terminal group and, especially, of the His and Tyr residues causes a slight but structurally extensive reorganization in the SL-AT molecule; at alkaline pH values the molecule becomes more compact.

3. DISCUSSION

The present approach thus leads, as in the case of cyclotuftsin [1], to a proper qualitative description of conformer equilibrium in solution. It is possible, for instance, to exclude structures drastically inconsistent with experimental data or, on the contrary, to select structures which are indispensable for reaching complete agreement with experimental evidence. It should be pointed out that since SL-AT possesses nearly the same affinity and specificity towards AT antibodies as AT, the above results may be attributed to the AT molecule as well. However one must realize that the obtained weights are only approximate estimates; the determination of possible variation limits for such values would be the subject of future investigation.

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