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Combined use of spectroscopic and energy calculation methods for the determination of peptide conformation in solution

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This paper describes the combined use of energy calculations and spectroscopic data for the determination of peptide conformations in solution. The approach involves (i) experimental measurements of spectroscopic parameters for a molecule, (ii) calculation of these parameters for low-energy conformers previously determined with regard to local fluctuations in conformation and (iii) a random search for statistically weighted combinations of conformers which provide a good agreement between the calculated and experimental data. The above approach was used to study the conformation of a spin-labelled angiotensin molecule (SL-AT). It appears that the C-terminal hexapeptide of SL-AT possesses two geometrically different spatial forms of the backbone in aqueous solution, with mean values for the statistical weight of 0.78 and 0.22, respectively. In contrast, the N-terminal part of the molecule is conformationally labile. The data obtained can be used to describe the conformation of angiotensin in solution.

1. Introduction

Physico-chemical experiments, in particular spectroscopic studies, provide the bulk of information useful for the description of the three-dimensional structure of peptides in solution. However, the interpretation of these data meets with serious difficulties, because experimentally measured parameters represent values averaged over the entire ensemble of molecular conformers existing in dynamic equilibrium in solution. As pointed out in ref. 1, under these circumstances, the 'averaged' peptide conformation deduced from spectroscopic data has no physical significance.

In fact, the characteristic rates of molecular motions for many oligopeptides in solution can be ranged as $\tau_s \ll \tau_c \ll \tau_i$, where τ_s is the lifetime of a conformer, τ_c the correlation time of the overall molecular motions and τ_i the time related to internal motions. As the molecular conformers in solution are spectroscopically indistinguishable, it is valid to assume that the characteristic time for an experiment would exceed τ_s considerably. Hence, the averaged value of the experimentally measured k-th parameter, $\langle A_k \rangle$, can be expressed as

$$\langle A_k \rangle = \sum_{i=1}^{N} w_i A_{ik},$$

where the w_i are the values of the statistical weights for the molecular conformers existing in solution.

Consequently, an adequate description of the spatial structures of peptides in solution is inconceivable without knowledge of the values of both the A_{ik} (i.e., the possible three-dimensional structure of conformers) and of the statistical weights, w_i. An approach to solving this problem, based on the combined use of experimental data and the results from energy calculations, has been outlined

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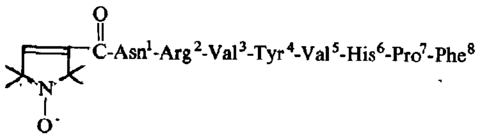
in our recent papers [2–4]. The computations involve selection of low-energy structures for the peptide backbone of the molecule by means of energy calculations, generation of statistical samples of conformations in the vicinity of each structure by the Monte-Carlo technique, and calculation of the mean values $\langle A_{ik}^{\text{calcd}}\rangle$ and their standard deviations D_{ik}^{calcd} for each sample. Further, by assuming that the values $\langle A_k^{\text{exp}}\rangle$ and $\sum_i w_i \langle A_{ik}^{\text{calcd}}\rangle$ are statistically indistinguishable according to the Student's coefficient chosen, w_i values are selected (using random search algorithms) providing 'good fit' conditions between experimental and calculated data:

$$\frac{\left|\sum_i w_i\langle A_{ik}^{\text{calcd}}\rangle - \langle A_k^{\text{exp}}\rangle\right|}{\left|\sum_i \left(w_i D_{ik}^{\text{calcd}}\right)^2 + \left(D_k^{\text{exp}}\right)^2\right|^{1/2}} \leqslant t_k, \qquad (1)$$

$$\sum_i w_i = 1; \quad w_i \geqslant 0$$

Ideally, the present approach requires experimental parameters allowing explicit interpretation in terms of the values of the dihedral angles or interatomic distances. However, the number of such parameters for peptides is usually confined to the values of vicinal coupling constants, depending on the dihedral angles [5], the efficiency of singlet-singlet energy transfer between aromatic moieties [6], and nuclear Overhauser effects, indicating proton–proton distances of less than 3–4 Å [7]. In this connection, NMR of spin-labelled peptides is a subject of particular interest, because the rate of spin-lattice proton relaxation induced by the unpaired electron of the spin label depends upon the sixth power of the distance between the proton and the electron [8]. Our previous works [2–4] as well as the present study are largely based on the use of this experimental approach. It should be borne in mind that this method has not been applied previously to conformationally labile molecules due to the difficulties involved in conformer averaging mentioned above.

The model molecule used in the present work was the same as that in refs. 2–4, namely, a spin-labelled derivative of angiotensin, a peptide bioregulator displaying a wide range of biological activities:



(SL-AT)

The τ_c value of this molecule (6.5×10^{-10} s) was derived from ^{13}C-NMR data [9], $\tau_s \geqslant 10^{-6}$ s (estimated in ref. 4 from calculation) and τ_i was equal to $10^{-10} \div 10^{-12}$ s [10,11]; hence, the conformational mobility model discussed above appears to be valid for SL-AT in aqueous solution.

The experimental procedures and methods of energy calculations have been described in detail elsewhere [4]. The spin-lattice relaxation rates $1/T_{\text{IM}}$ for C$^\alpha$H Val^3, C$^\delta$H Tyr^4, C$^\epsilon$H Tyr^4, C$^\alpha$H Val^5, C$^\delta$H His^6*, C$^\epsilon$H His^6, C$^\beta$H Pro^7, C$^\delta$H Pro^7 and C$^\epsilon$H Phe^8 protons as well as the vicinal coupling constants J(HNC$^\alpha$H) and the value of singlet-singlet energy-transfer efficiency T between Phe^8 and Tyr^4 residues were measured for SL-AT in ^{2}H$_2$O (or H$_2$O) at different pH values (pH 8.3, 4.0 and 2.3). Low-energy conformers of 12 types were analyzed with respect to the SL-AT peptide backbone; statistical samples, each comprising 15 000 conformations, were generated in their vicinity. The aqueous environment was partially modelled during energy calculations by an appropriate choice of the value of the dielectric constant [12]. The mean values of $1/T_{\text{IM}}$, J, T and their standard deviations were then calculated for each sample, taking into account their dependence on dihedral angles and interatomic distances; correlation functions were used to make provisions for rapid internal motions related to the $\langle 1/T_{\text{IM}}\rangle$ values.

Sets of statistical weights (i.e., 12-dimensional points $\{w_i\}$) were found for SL-AT conformers using a random search algorithm for each of the three experimental data variants corresponding to different pH values. Every set comprised more than 500 $\{w_i\}$ points, each point satisfying the conditions (eq. 1), with all t_k values being less

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* Data for pH 2.3 and 4.0 are not available.

Table 1

Limits of statistical weights for SL-AT conformers

A dash denotes the absence of a statistically reliable w_i^* value.

pH	Statistical weight (%)	Conformer no.											
		1	2	3	4	5	6	7	8	9	10	11	12
2.3	$w_{i\,\min}$	0	0	0	2	6	0	0	4	0	0	0	9
	$w^*_{i\,\min}$	8	–	–	3	–	14	–	5	–	–	2	–
	$w^*_{i\,\max}$	–	6	–	–	25	–	1	–	–	2	14	20
	$w_{i\,\max}$	30	18	14	17	36	22	6	9	19	3	24	23
4.0	$w_{i\,\min}$	1	0	0	1	0	0	0	5	0	0	0	1
	$w^*_{i\,\min}$	–	–	–	–	8	14	–	–	–	–	–	12
	$w^*_{i\,\max}$	24	5	–	–	–	–	2	9	14	–	13	–
	$w_{i\,\max}$	32	22	18	16	31	28	6	13	26	2	30	19
8.3	$w_{i\,\min}$	5	0	0	1	0	6	0	5	0	0	0	5
	$w^*_{i\,\min}$	24	–	5	5	–	20	–	–	–	–	–	11
	$w^*_{i\,\max}$	–	3	–	–	–	–	2	–	9	1	4	–
	$w_{i\,\max}$	36	11	19	19	12	31	6	13	34	3	21	17

than 1.96; in other words, each point provides agreement between the experimental and calculated parameters to within confidence limits of 95%. In all three cases, the $\{w_i\}$ points obtained form single convex sets on the normalization hyperplane and the general features of the sets do not change upon further increase in the number of points; this applies, for example, to the possible maximum and minimum limits of conformers' statistical weights w_i. Clearly, the sets of points $\{w_i\}$ vary according to the type and number of experimental parameters contributing to the conditions (eq. 1). In this respect, the proposed approach to investigation of the spatial structure of conformationally labile molecules in solution actually represents an algorithm for progressive refinement of statistical weights of conformers during the course of experimental data accumulation.

The values of $w_{i\,\min}$ and $w_{i\,\max}$ are summarized in table 1. Several conformers possessing nonzero lower limits to the w_i values are considered to be 'indispensible' for obtaining good agreement between experimental and calculated data, namely, structures 1, 4, 6, 8 and 12 at pH 8. 3, structures 1, 4, 8 and 12 at pH 4.0 and structures 4, 5, 8 and 12 at pH 2.3. Nevertheless, the good fit conditions eq. 1 cannot be satisfied using only the indispensible conformers (for details, see ref. 3). It should also be noted that the distributions of w_i values over the sets of $\{w_i\}$ points are nonuniform; this permits lowering of the upper limit to the statistical weights for some conformers to $w^*_{i\max}$ (or raising of the lower limit to $w^*_{i\min}$) without significantly affecting the agreement obtained between experimental and calculated parameters. The $w^*_{i\min}$ and $w^*_{i\max}$ values are also listed in table 1; these were determined by comparison of a sample of $(\Sigma_k\ t_k^2)^{1/2}$ values for the distribution of w_i over the entire set of $\{w_i\}$ points with the same sample being limited according to the condition $w_i \geqslant w^*_{i\min}$ (or $w_i \leqslant w^*_{i\max}$). This comparison was performed using the methods of non-parametrical statistics (Wilcoxon's criterion) [13].

The mean values for w_i of conformers' statistical weights can also be deduced from the sets of $\{w_i\}$ points obtained in this way. It should be pointed out in this connection that conformers corresponding to different local energy minima within the space of the dihedral angles may sometimes possess a geometrically similar organization of the peptide backbone. The geometrical similarity shared by backbone conformer pairs of SL-AT was assessed in the present study by calculating the deviation D for the best-fit case (according to the algorithm described in ref. 14 for the spatial arrangement of the C^α and C^β atoms when the

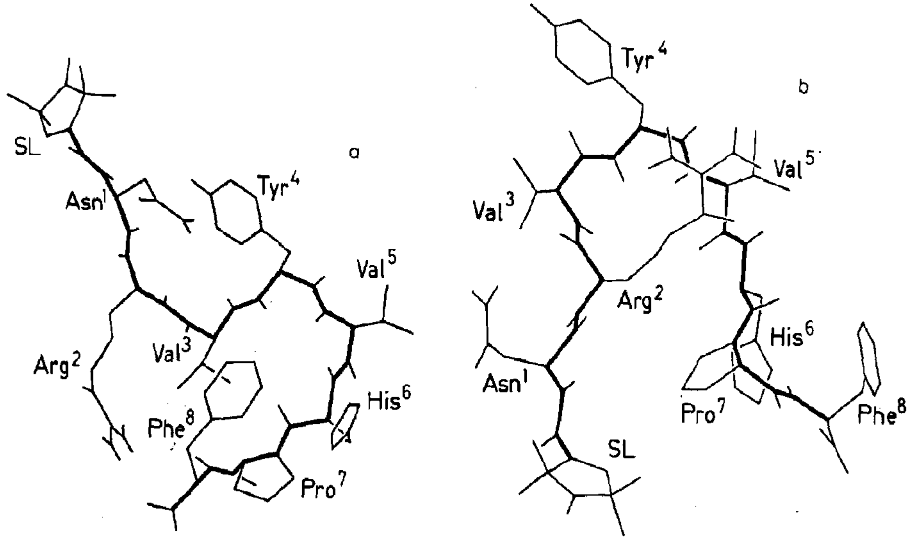

Fig. 1. SL-AT structures with a β-turn centred on the Tyr^4-Val^5 (a, structure I in table 2), or Val^3-Tyr^4 region (b, structure V).

two conformers are superimposed. The value of D was calculated using the following expression:

$$D = \tfrac{1}{16}\sum_{i=1}^{16}\left[(x_i - x_i')^2 + (y_i - y_i')^2 + (z_i - z_i')^2\right],$$

where x, y and z are the Cartesian atomic coordinates, the prime ($'$) denoting the second conformer in each pair. The conformers in a pair were regarded as similar when $D \leqslant 1.0$ Å^2. The above-described assessment of the geometrical similarity between conformers together with examination of the distributions of intramolecular distances between C^α atoms for all SL-AT conformers permits a rough division of SL-AT backbone conformers

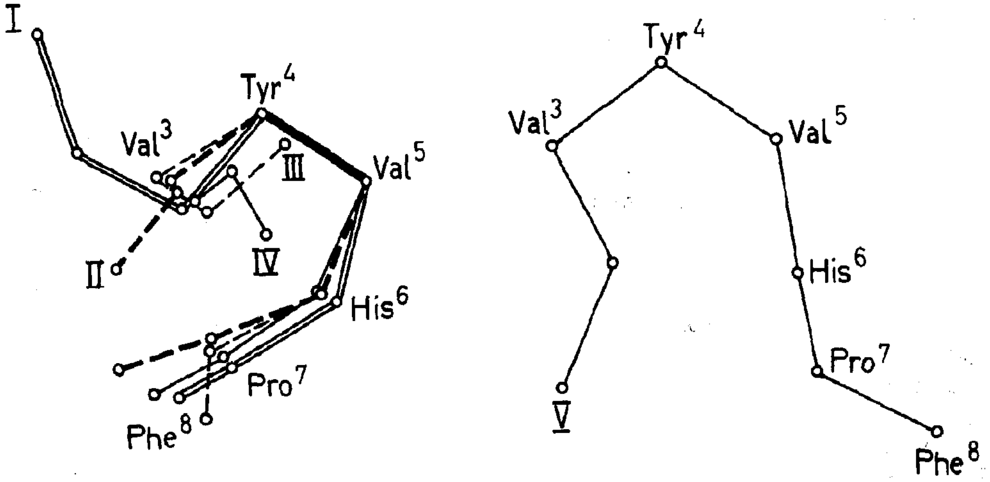

Fig. 2. Schematic representation of the spatial arrangement of C^α atoms for different types of SL-AT peptide backbone (numbered according to table 2).

Table 2

Geometrically different types of SL-AT backbone structures

Structural type	SL-AT backbone conformers	Statistical weight of structure type (%)	Interresidue distance (Å)			
			R_{1-6}	R_{3-6}	R_{3-8}	R_{1-8}
I	1, 9	0.350	12.15	5.63	6.84	12.30
II	6	0.191	9.35	5.78	5.68	6.79
III	2, 4, 11	0.140	7.40	5.60	6.84	9.49
IV	3	0.048	8.19	5.22	5.70	8.54
V	8, 12	0.215	7.98	7.66	12.92	10.74

into several types of geometrically different structures.

Structural types with a mean statistical weight greater than 0.045 are detailed in table 2. This table summarizes the mean distances between the C^{α} atoms of Asn^1, Val^3, His^6 and Phe^8, indicating the several variations in central and terminal parts of the peptide backbone geometry. The data in table 2 suggest that the central part of the molecule is conformationally the most rigid. A distinct β-III turn [15] is centred either on the Tyr^4-Val^5 region (mean statistical weight, 0.78 over all conformers; structures I–IV, table 2) or on the Val^3-Tyr^4 region (mean statistical weight, 0.22; structure V). The same backbone geometry variants are retained in the C-terminal hexapeptide (fig. 1 shows two SL-AT conformers corresponding to structure I and V). In contrast, the N-terminal part of the molecule shows high conformational lability: all of the main types of geometrically different backbone structures are depicted schematically in fig. 2.

The values of the mean statistical weights given above were obtained at pH 8.3. It appears from data in table 1 that the dynamic equilibrium between SL-AT conformers in solution is affected by changes in pH; it would be even more prone to variation as a result of amino acid substitutions or following elimination of the spin label (possible changes in statistical samples of AT conformers induced by amino acid substitutions in positions 1, 4 and 8 have been discussed in ref. 4). Hence, the question arises as to whether the above results obtained on SL-AT can be extended to the three-dimensional structure of angiotensin in solution which has been investigated by many authors.

Indirect evidence in favour of a definite similarity in spatial structure between SL-AT and AT is provided by the observation that SL-AT possesses the same affinity towards anti-AT antibodies as AT itself. This may be due to the conformational rigidity of the central part of the peptide backbone (Val^3-His^6 fragment) discussed above, which is largely responsible for the specificity of AT binding [16]. Calculations also demonstrate that geometrical similarity exists between the same low-energy backbone conformers of SL-AT and AT (estimated using the above algorithm with $D \leqslant 1.0$ Å^2). However, the main conclusions as to the SL-AT and AT conformers existing in aqueous solution can apparently be made only on the basis of the physico-chemical parameters of AT, allowing more or less explicit conformational interpretation.

Unfortunately, such data as are available in the literature are sometimes contradictory and, in general, of little use (for reviews, see refs. 17 and 18). For instance, good agreement between the values of $J(HNC^{\alpha}H)$ measured for SL-AT and AT [19] does not demonstrate similarity in the overall spatial organization of both molecules, since the vicinal coupling constants are local structural parameters. Nevertheless, there is good coincidence between the mean radius estimates for AT: the experimental estimate is $r \leqslant 8$ Å [20], the calculated value being $r = 6.6$ Å (the latter was obtained by calculating the average radius of inertia of SL-AT (assuming the absence of spin label and using the mean values of statistical weights w_i). Results obtained from titration of some functional groups in AT at different pH values can be interpreted in terms of the average distances between groups [21]: the data in table 3 indicate that experimental findings with AT are not in contradiction with parameters calculated for SL-AT. Data on NMR and isotopic exchange kinetics for AT reviewed in ref. 17 suggest that the amide proton of Arg^2 is exposed to the solvent to a greater extent than amide protons of the C-terminal hexapeptide, one or two of which are of the slow type. On the other hand, estimation of the average 'surfaces' of SL-AT amide protons accessible to solvent (calcula-

Table 3

Several mean interatomic distances with the SL-AT molecule

Interatomic distances were calculated using the mean statistical weights of SL-AT conformers corresponding to pH values close to those obtained experimentally [21].

Distance	Calculated (Å)	Experiment [21] (Å)
αNH_3–COO^-	10.5–10.7	9–10
Asp^1–COO^-	9.5– 9.7	9–10
His^6–COO^-	7.4– 7.6	8– 9
Asp^1–His^6	9.1– 9.3	8– 9

tion procedure according to the algorithm from ref. 22) resulted in the following relative values for the 'accessible surfaces' at pH 8.3: 1.0 for NH Arg^2, 0.39 for Val^3, 0.08 for Tyr^4, 0.31 for Val^5, 0.18 for His^6 and 0.53 for Phe^8. Finally several experimental papers reviewed in ref. 23 reported the complexing of heavy metal ions (La^{3+} or Gd^{3+}) with the Asp^1 β-carbonyl and C-terminal carboxyl of the AT molecule, allowing postulation of the close proximity of these functional groups in solution. The calculated $C^{\alpha}_{Asn^1}$–$C^{\alpha}_{Phe^8}$ distances shown in table 2 also support the possibility of ion complexation by the terminal residues of SL-AT, since the end-to-end distance is rather small in several SL-AT conformers (e.g., structure II). At the same time, low values of $d\delta/dT$ for the amide proton of His^6 also observed in ref. 23 can be interpreted in terms of proton shielding by the solvent (see calculated estimates above) instead of the involvement of the proton in intramolecular hydrogen bonding as suggested in ref. 23.

Generally speaking, it can be concluded that the characteristics determined on SL-AT conformers in aqueous solution in the present studies do not contradict experimental data available on angiotensin and, therefore, that SL-AT can be used, to a certain extent, to describe the dynamic equilibrium of AT conformers in solution. It is hoped in the future to determine the nuclear Overhauser effects for AT or SL-AT in aqueous solution; the present work allows averaging of the corresponding functions, depending on proton–proton distances and prediction of intramolecular effects between the following protons of non-adjacent residues: NH Val^3-$C^{\epsilon}H$ Phe^8, $C^{\alpha}H$ Val^3-$C^{\epsilon}H$ Phe^8, and NH Tyr^4-NH His^6.

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