CD spectrum and conformational distribution of cyclotuftsin in solution

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CD spectra for low-energy conformations of the tuftsin cycloanalogue, Thr-Lys-Pro-Arg-, were calculated. A theoretical spectrum obtained as the weighted average of calculated spectra for individual peptide backbone conformers is qualitatively consistent with an experimental CD spectrum in aqueous solution. The conformational distribution allows one to achieve agreement between calculated and experimental values of structural parameters of the cyclotuftsin molecule investigated by NMR spectroscopy.

CD spectrum calculation *Theoretical conformational analysis* *Tuftsin cycloanalog* *Peptide conformation*

1. INTRODUCTION

Experimentally measured CD spectra, even for conformationally restricted peptides, are the sums of contributions of various molecular conformers existing in solution. Therefore, interpretation of the CD spectrum for a given peptide in conformational terms would inevitably involve data on potentially stable molecular space structures (e.g., obtained by means of energy calculations) with subsequent calculation of the corresponding CD spectra and evaluation of statistical weights for each particular conformer. A similar procedure carried out earlier on model compounds (e.g. [1–3]) has revealed considerable differences between experimental CD spectra and the averages of calculated spectra for individual conformers. In our view, this discrepancy is mainly due to the use of Boltzmann statistical weight estimates for conformer weights in [1–3]. Errors inherent in the conformational energy calculations (neglect of entropy factor, solvent effects, etc.) lead to considerable uncertainties in weight estimations. It appears more reasonable to determine conformer weights using fitting of the experimental CD spectrum by weighted averages of calculated CD spectra for low-energy structures. This work reports such an attempt for the biologically active tuftsin cycloanalogue, Thr-Lys-Pro-Arg-, examined by us earlier [4].

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2. METHODS AND RESULTS

Energy calculations for the cyclotuftsin molecule were essentially the same as in [4], but using a system of interatomic potentials and other parameters proposed in [5]. As results, 14 low-energy conformations were selected ($\Delta U \leqslant$ 7.8 kcal/mol, as in [4]). These conformations can be arranged into 5 groups according to the signs of the dihedral angles responsible for the mutual spatial orientation of the peptide chromophores: BBRB, BLRB, BBRR, BLRR and RBBL containing 3, 4, 5, 1 and 1 structures, respectively (table 1). CD spectra were calculated for each of the 14 conformers by the procedure in [6] and using the calculation scheme adopted in [1–3]. Thereby, only the $n\pi^*$ and $\pi\pi^*$ transitions of the peptide chromophores were taken into account with the initial parameters of Gaussian bands (peak position λ_o and half-band width Δ_o), λ_o = 215 nm, Δ_o = 13.4 nm and λ_o = 187 nm, Δ_o = 14 nm, respective-

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Table 1

The principal groups of low-energy conformations of the Thr-Lys-Pro-Arg-peptide backbone[a]

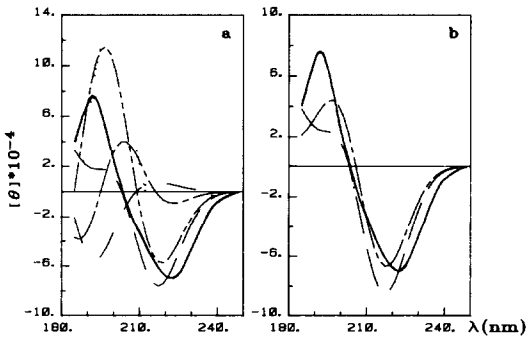
Residue	Angle	Backbone structures[b]				
		BBRB	BLRB	BBRR	BLRR	RBBL
Thr	ω	180	180 ± 1	180	−179	177
Lys	ϕ	−150 ± 1 (−149)	60 ± 1 (59)	−121 ± 9 (−130)	63	−155
	ψ	110 ± 25 (86)	89 ± 4 (85)	128 ± 19 (109)	148	157
	ω	−174 ± 4 (−171)	−175 ± 15 (−171)	−165 ± 21 (−174)	144	177
Pro	ψ	−47 ± 8 (−54)	−41 ± 13 (−54)	−45 ± 4 (−41)	−50	100
	ω	165 ± 5 (161)	166 ± 6 (161)	177 ± 10 (167)	−173	−146
Arg	ϕ	−126 ± 5 (−121)	−118 ± 3 (−121)	−106 ± 9 (−115)	−97	50
	ψ	74 ± 6 (80)	46 ± 31 (77)	−49 ± 7 (−56)	−63	75
	ω	−160 ± 5 (−155)	−176 ± 21 (−155)	164 ± 4 (168)	160	−165
Weights of structural groups[c]						
(1)	W_i	0.66	0.00	0.30	0.00	0.04
(2)	W_i	0.99	0.01	0.00	0.00	0.00

[a] Average angles and their deviations are indicated for each group of structures. The values corresponding to lowest-energy structures for each group are indicated in parentheses

[b] Designations of potential map quadrants for the dipeptide unit: B, $\phi < 0°$, $\psi > 0°$; R, $\phi < 0°$, $\psi < 0°$; L, $\phi > 0°$, $\psi > 0°$ (reference system as in [7])

[c] (1) $[\theta(\lambda)]_i$ selected as curves of fig.1a; (2) Boltzmann estimates, T = 300 K

ly [1] (for a tertiary amide λ_o = 198 nm, Δ_o = 14 nm). Other parameters required for computations were chosen as in [1,2,6].



It appears that the calculated CD spectra share a certain similarity within each particular conformer group of table 1. The spectral characteristics of each group are shown in fig.1a. Statistical weight estimates for each group (W_i)

Fig.1. CD spectra of Thr-Lys-Pro-Arg-: experimental spectrum in water [4] (——); (a) the arithmetical averages of calculated spectra for each conformer group: BBRB (– – –), BLRB (– – –), BBRR (– – – –), BLRR (·····) and RBBL (–·••–); (b) weighted averages of calculated spectra according to the obtained set of weights (– – –) and Boltzmann weights (–·••–); molar ellipticity $[\theta]$ in degree/cm^2 per dmol.

Table 2

Comparison of calculated and experimental structural parameters for the Thr-Lys-Pro-Arg-] molecule[a]

Structural parameters	Averaged estimates	Experiment [4]	
		Water	DMSO
Coupling constants $J(HNC^{\alpha}H)$ (Hz)[b]			
NH (Lys)	8.3 ± 0.9	–	8.2
NH (Arg)	10.5 ± 0.4	9.9	10.1
$N^{\epsilon}H$ (Lys)	12.0 ± 1.6	12.5	11.4
Chemical shift differences $\Delta\delta^{\beta,\gamma}_{Pro}$ (ppm)[c]	4.59 ± 0.20	4.13	–

[a] Deviation of calculated parameters is due to deviation of dihedral angle values in table 1

[b] Calculated as in [8]; experimental J values corrected according to [8]

[c] Calculated as in [9]

were determined by means of an iterative computational procedure, minimizing the root-mean-square deviation between the experimental CD spectrum in aqueous solution [4] and the weighted average of calculated spectra ($\sum_i W_i[\theta(\lambda)]_i$) for λ values ranging from 185 to 245 nm with $\sum_i W_i = 1$ and $W_i \geqslant 0$ restrictions.

The curves in fig.1a were chosen as $[\theta(\lambda)]_i$ terms. The resultant set of W_i is indicated in the bottom line of table 1, and the corresponding averaged CD spectrum depicted in fig.1b.

3. DISCUSSION

The calculated average CD spectrum in fig.1b closely resembles the experimental one. The described procedure rejects only the BLRB and BLRR structures. The same results have been obtained with spectra calculated for lowest-energy conformations in each group chosen as $[\theta(\lambda)]_i$ terms. For the remaining structures there is apparently a whole range of equally acceptable W_i values. At the same time, it is evident that the averaging procedure performed with calculated Boltzmann weights yields an average CD spectrum resembling the experimental one only slightly (fig.1b).

The averaging of some structural parameters of cyclotuftsin with the W_i set demonstrates reasonable consistency with experimental values determined previously by NMR spectroscopy [4] (table 2).

Structures BBRB and BLRB have been proposed as potential biologically active cyclotuftsin conformations in [4]; it follows from table 1 that the weight of only one of these structures in solution is not equal to zero.

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