CONFORMATION OF A SYNTHETIC 34-RESIDUE POLYPEPTIDE THAT INTERACTS WITH NUCLEIC ACIDS

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1. Introduction

In [1] the design, synthesis and properties of a 34-residue polypeptide with ribonuclease activity were studied. Based on secondary structure prediction rules [2] and model building, an attempt was made to build a three-dimensional configuration capable to bind a trinucleotide.

The amino acid sequence is given in table 1. In the sequence an intrachain disulfide bridge between half-cystines 10 and 33 is intended to stabilize the structure, and to bring potential active site residues (Asp^{7}, Thr^{12}, His^{32}) into spatial proximity [1]. In addition to the given 34-residue polypeptide, a 68-residue dimer and higher oligomers with interchain cystine bridges are obtained. The respective [Ala^{10}, Ala^{33}]-analogue which cannot form disulfide bonds was prepared as a reference, in order to investigate the role of the covalent crosslink in stabilizing the peptide fold.

As shown by difference spectra and enzymatic assays, the 3 peptides all interact with cytidine phosphate or single-stranded DNA, showing significant ribonuclease activity with preference for cleavage at the 3′-end of cytidylate. The nuclease activity of all 3 compounds suggests that the disulfide bond is not essential for the structure and function; obviously the non-covalent interactions are sufficient to establish a structure capable to bind and cleave the substrate [1]. The present measurements are intended to characterize the 3 peptides by CD spectroscopy in order to prove or disprove the structure predicted on the basis of statistical and stereochemical methods.

As a result the far-ultraviolet spectra of the 3 compounds all exhibit characteristic minima at 225 and 198 nm reflecting a considerable contribution of β-structure but disproving the presence of significant amounts of helix. The spectra are also very similar in the range of aromatic transitions (260–290 nm), pointing to anomalous absorption of the Phe and Tyr residues. CD spectral titration of the 68-residue polypeptide with 2′-CMP lends itself to verify the binding constant determined by chromatographic techniques. K_a was found to be $2.0 \times 10^5\ M^{-1}$.

Table 1
Amino acid sequence and predicted secondary structure of the 34-residue polypeptide

	1				5				10				15				20				25				30				
	F	T	F	T	Y	T	D	P	N	C	Q	T	G	Q	G	Q	N	P	N	G	I	S	E	P	T	A	A	K	V	Q	A	H	C	A
Lim [5]	β	β	β	β	β																							β	β	β	β	β			
Chou/Fasman [6]	β	β	β	β	β		τ	τ	τ	τ							τ	τ	τ	τ			α	α	α	α	α	α	α	α	α	α	α	
Burgess et al. [7]							τ	τ	τ	τ							τ	τ	τ	τ					α	α	α	α	α					
Average	β	β	β	β	β		τ	τ	τ	τ							τ	τ	τ	τ					α	α	α	α	α					

2. Materials and methods

The solid-phase synthesis and purification of the 34- and 68-residue polypeptides and their [Ala[10], Ala[33]]-analogue have been reported [1]. Relevant physical parameters are given in table 2. For the present spectroscopic analysis solvent conditions were chosen as in the previous absorption studies. Chemicals were of A-grade purity (Merck, p.a.; Schwarz-Mann, 'ultrapure'). Bidistilled water was used throughout.

Concentration determination was based on dry weight or on the absorption at 275 nm (cf. table 2), using Cary 118 and Zeiss DMR 10 spectrophotometers. Circular dichroism measurements were performed in a Roussel-Jouan Dichrographe II with high-sensitivity equipment, and a Cary 61 CD-spectrometer, using 0.1–10 mm cuvettes. To measure 2′-CMP binding, spectral titrations made use of the near UV extrinsic Cotton effect, applying a microburet (Hormuth and Vetter), and correcting for dilution. As reference spectra for main-chain conformations those in [3] were used. For secondary structure prediction from the amino acid sequence 3 different methods were computerized [4]. The original methods are detailed in [5–7]. Concerning the Chou and Fasman approach [6] single residue probabilities were used. β-Turns predicted with the algorithms given in [6] and/or [7] were accepted only if they coincided in position with a relative minimum in the hydrophobicity profile [8]. For increased reliability the results of the single approaches were averaged. Predicted α-helix and β-structure were discarded if shorter than 4 and 3 residues, respectively.

3. Results and discussion

As outlined [1] the 34-residue polypeptide is expected to contain a β-stranded core at its N-terminus (residues 1–6 and 11–16) interrupted and followed by turns (residues 7–10 and 17–20), and an α-helical segment at the C-terminus (residues 23–33). Extension of secondary structure prediction to include different approaches and recent improved parameters modifies the picture to a certain extent (see table 1). While the N-terminal stretch of β-structure (residues 1–5) and the β-turns (residues 7–10 and 17–20) are further substantiated, there is no support for β-structure over the positions 11–16, and the helical segment at the C-terminus appears to be shorter as well as in part questioned by a rather high β-potential (table 1, and fig.1, cf. [5]).

CD spectra were calculated with the standard data [3] for the secondary structure compositions resulting from the average and from the Chou-Fasman pre-

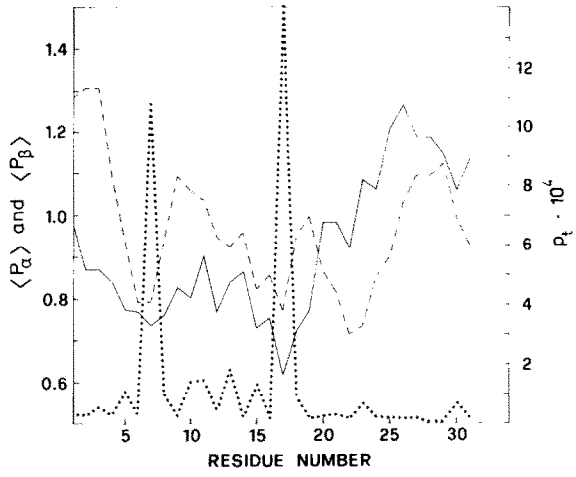

Fig.1. Conformational profiles predicted with the method of Chou and Fasman [6]: (——) average α-helical potential $\langle P_\alpha \rangle$; (– – – –) average β-structure potential $\langle P_\beta \rangle$; (· · · ·) β-turn probability p_t.

Table 2
Characterization of the polypeptides (cf. [1])

	M	$E^{a}_{275\ nm}$	$K_{a,CMP}$ (M^{-1})	A^{b}_{rel}
34 [Cys[10], Cys[33]]	3596	3480	6.7×10^3	0.1
34 [Ala[10], Ala[33]]	3534	2880	$< 1 \times 10^3$	<0.1
68 SS dimer	7192	3200	2.0×10^5	2.5

[a] Molar absorption at 275 nm based on the molecular weight of the 34-residue polypeptide
[b] Relative nuclease activity, compared to natural RNase A (≡ 100)

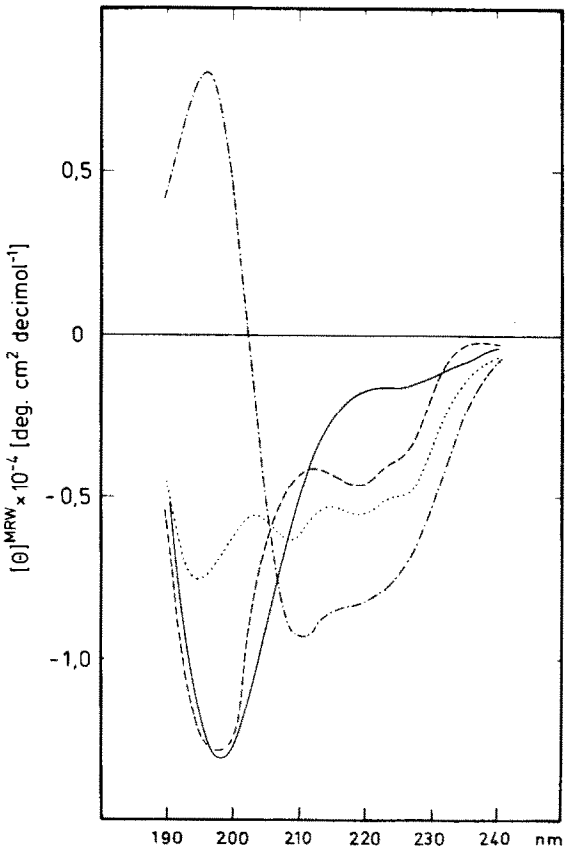

Fig.2. Observed, fitted and predicted CD spectra of the 34-residue polypeptide [Cys^{10}, Cys^{33}]: (——) observed in 0.005 M phosphate buffer pH 6.5; (– – – –) least squares fit with standard data given in [3]; (– · – · –) calculated with standard data [3] for the secondary structure pattern predicted according to Chou and Fasman [6]; (· · · ·) calculated for the pattern corresponding to the average prediction from table 2.

diction (table 1) as the design of the peptide has been based on the latter. The calculated spectra can be compared in fig.2 with the observed spectrum and that representing the least squares fit. The optimum fit corresponds to a combination of 0% (0 residues) helix, 39% (13 residues) β-structure, 12% (4 residues) β-turn and 49% (17 residues) random coil. In order to check how this result is affected by errors in concentration (which tends to be too high rather than too low), the analysis was repeated for the same spectrum assuming the concentration to be only 75% of the nominal value. The only effect is that the portion of β-structure drops to 26% (9 residues).

The residual misfit in the wavelength range 210–

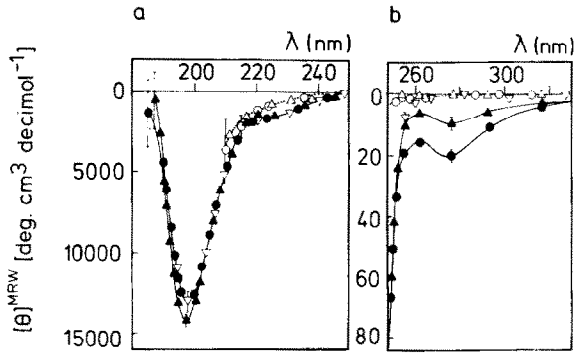

Fig.3. Far UV (a) and near UV (b) CD spectra of the 34-residue polypeptides [Cys^{10}, Cys^{33}] (●,○) and [Ala^{10}, Ala^{33}] (▲,△,▽), 0.01 M Na-citrate (pH 5.3), 0.01 M Na-acetate (pH 5.3), or 0.005 M Na-phosphate (pH 6.5); c = 0.03–1.0 mM, 20°C. Full line and closed symbols, 'native' polypeptides; dotted line and open symbols, denatured in the presence of 6 M guanidine · HCl (○,△); 1% SDS (▽).

230 nm could in principle be due to positive aromatic contributions. This possibility, however, is ruled out by the observation that 1% sodium dodecylsulfate (SDS) abolishes the aromatic bands in the near UV without affecting the spectrum in the peptide region (see fig.3,4a). Another explanation comes from the

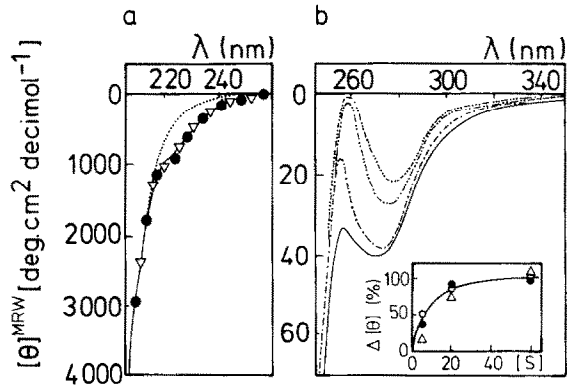

Fig.4. Far UV (a) and near UV (b) CD spectra of the 68-residue dimer. 0.01 M Na-acetate (pH 5.3); c = 8.8 μM, 20°C. (a) 'Native' polypeptide in the absence (full line), and presence of saturating concentrations of 2'-CMP (●, 20 μM), 1% SDS (▽), and 6 M guanidine · HCl (dotted line), respectively. (b) CD spectral titration with 2'-CMP: 0 μM (——); 6 μM (– –); 20 μM (– · –); 60 μM (– · · –); corrected for the intrinsic ellipticity of the ligand 2'-CMP. Insert: 2'-CMP binding curve as taken from the extrinsic Cotton effect at 257 (○), 275 (△), 300 nm (●), respectively; [S], 2'-CMP concentration in μM.

fact that the standard data are extracted from the X-ray structures of higher molecular weight globular proteins and may have reduced validity in the case of rather small polypeptides [9]; the same applies to the statistical parameters for structure prediction. Though the figures given above should be taken with care, it is obvious that the observed spectrum is incompatible with the amount of helix predicted with the method of Chou and Fasman and even so with the reduced amount surviving the averaging in table 1. It is noteworthy that the amount of β-structure predicted by the Lim algorithm is in good agreement with the value resulting from the least squares spectral fit.

The realization of a conformation potential can sometimes be stimulated by addition of SDS [10]. However, there is no helix-promoting effect of SDS on either the 34-residue polypeptide or the 68-residue dimer; on the other hand, significant structural changes are indicated in the range of aromatic absorption (fig.3,4a). Strong denaturants like 6 M guanidine · HCl cause all characteristic features of the spectra to disappear, in accordance with the expected breakdown of the 'native' structure. This effect holds especially for the near UV.

Titration of the polypeptide with 2′-CMP strongly affects the near UV part of the spectrum while the far UV spectrum remains unchanged within the range of error (fig.4). The extrinsic Cotton effect in the near UV may be utilized to determine the binding constant of the 'substrate'. The titration was carried out with the 68-residue dimer which shows the highest relative nuclease activity (cf. table 2). The maximum change in ellipticity takes place at ~260 and 280 nm; the partition of the total spectral effect between the nucleotide and the aromatic side-chains (probably involved in the binding interactions) remains unresolved. The binding constant is found to be $K = 2.0 \times 10^5\ M^{-1}$ which is in agreement with the value obtained by chromatographic techniques ([1], cf. table 2).

The fact that the nuclease activity of the 68-residue dimer considerably exceeds that of the 34-residue polypeptides cannot be correlated with correspondingly pronounced differences in the spectra of the 3 polypeptides. However, the amplitudes of the near-UV bands seem to parallel the relative activities (cf. fig.4b and table 2).

The findings reported here can be summarized as follows:

The two monomeric and the dimeric forms of the basic 34-residue sequence investigated, all exhibit the same type of CD spectrum. According to a least squares fit with spectral standard data it is compatible with some 30–40% β-structure and a β-turn whereas the existence of α-helix is ruled out. The intra- and intermolecular fixation of the cysteine residues as well as their substitution in the [Ala^{10}, Ala^{33}]-analogue does not induce significant changes in the ordered backbone conformation. 1% SDS uncouples the interactions generating the optical activity of the near UV transitions of the aromatic side-chains. There is no concomitant destructive influence on the secondary structure existing nor an effect promoting the realization of helix potential.

The ellipticity of the near UV band of the 3 samples roughly follows their relative nuclease activity. The binding constant of the 68-residue dimer for 2′-CMP as determined by spectral titration of its extrinsic Cotton effect in the near UV, is $K = 2.0 \times 10^5\ M^{-1}$, in accordance with the result of other experimental techniques.

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