NMR STUDY OF THE MAIN COMPONENTS OF CLUPEINE AND THEIR POSSIBLE INTERACTION WITH NUCLEIC ACIDS

Patrick COZZONE, Claudio TONIOLO+ and Oleg JARDETZKY*

Institut de Chimie Biologique, Universite d'Aix-Marseille, Place V. Hugo, 13331 Marseille, Cedex 3, France, +Centro di Studi sui Biopolimeri, CNR, Instituto de Chimica Organica, Via Marzolo, 1-35100 Padova, Italy and **Stanford Magnetic Resonance Laboratory, Stanford University, Stanford, CA 94305, USA*

Received 7 October 1979
Revised version received 30 November 1979

1. Introduction

Clupeines are mono-protomines found in large quantities in herring sperm, usually complexed with DNA. The structure of these complexes and their functional role are not known, but their existence suggests that they may be of importance in regulating the expression of genetic information. Three principal components, YI, YII and Z of clupeine have been isolated, their sequence determined and their physical properties studied by CD and ^{13}C NMR [1–3]. The physical studies indicate that the polypeptides exist in aqueous solution predominantly in a random coil form, although some spectral changes suggest the formation of a partially helical structure in the presence of certain ions, such as phosphate and perchlorate. The present study was undertaken in the hope of clarifying additional structural features and the interaction with nucleic acid derivatives. The results are in agreement with the earlier conclusion that the polypeptides are random coils and that helix formation is not induced by non-specific binding to either nucleotide phosphates, single-stranded polynucleotides or short double-strand oligo [d(A–T)]. The pK values of the N-terminal amino groups have been obtained.

2. Experimental

The three clupeines YI, YII and Z have been prepared and characterized as in [1,2]. All ^1H NMR measurements were made on the modified Bruker HXS-360 NMR Spectrometer at the Stanford Magnetic Resonance Laboratory. ^{31}P measurements were made on a modified Varian XL-100 Spectrometer equipped with a Nicolet TT 1010A accessory and Nicolet Mona variable frequency probe [4].

3. Results

The 360 MHz ^1H NMR spectra of the two best characterized clupeines, YI and YII, are shown in fig.1. YI contains 2 Ala, 20 Arg, 1 Gly, 1 Ile, 2 Pro, 3 Ser, 2 Thr. YII contains 2 Ala, 20 Arg, 3 Pro, 2 Ser, 1 Thr, 2 Val. The assignments indicated in the figure were made by systematic homonuclear spin decoupling and comparison with the spectra of the free amino acids [4]. The β-CH_2 and γ-CH_2 resonances of the 20 arginine residues are the most predominant feature of the spectra, but cannot be resolved individually, even at high temperature. The Gly 27 and Ile 11 resonances in YI and the Thr 5 resonance in YII are assigned unequivocally, since only one such residue occurs in the peptide. Unequivocal assignment of Ala 1 and, by exclusion, of Ala 26 in YI and Pro 1 in YII is also possible, since the shifts of the N-terminal residues show a pH dependence when the amino (resp., imino) group is titrated. For the remaining resonances of residues present in groups of 2 or 3 in each peptide (Ser 6,7,8; Pro 10,16; Thr 20,21 in YI; Ala 8,26; Ser 9,21; Pro 11,27 and Val 12,20 in YII), only pairwise joint assignments are possible, since the resonances overlap. The only exceptions are Ala 8,26 in YII, where a slight shift is observed, and it can be argued that the lower doublet represents Ala 26 placed between two charged Arg 25,27 residues and

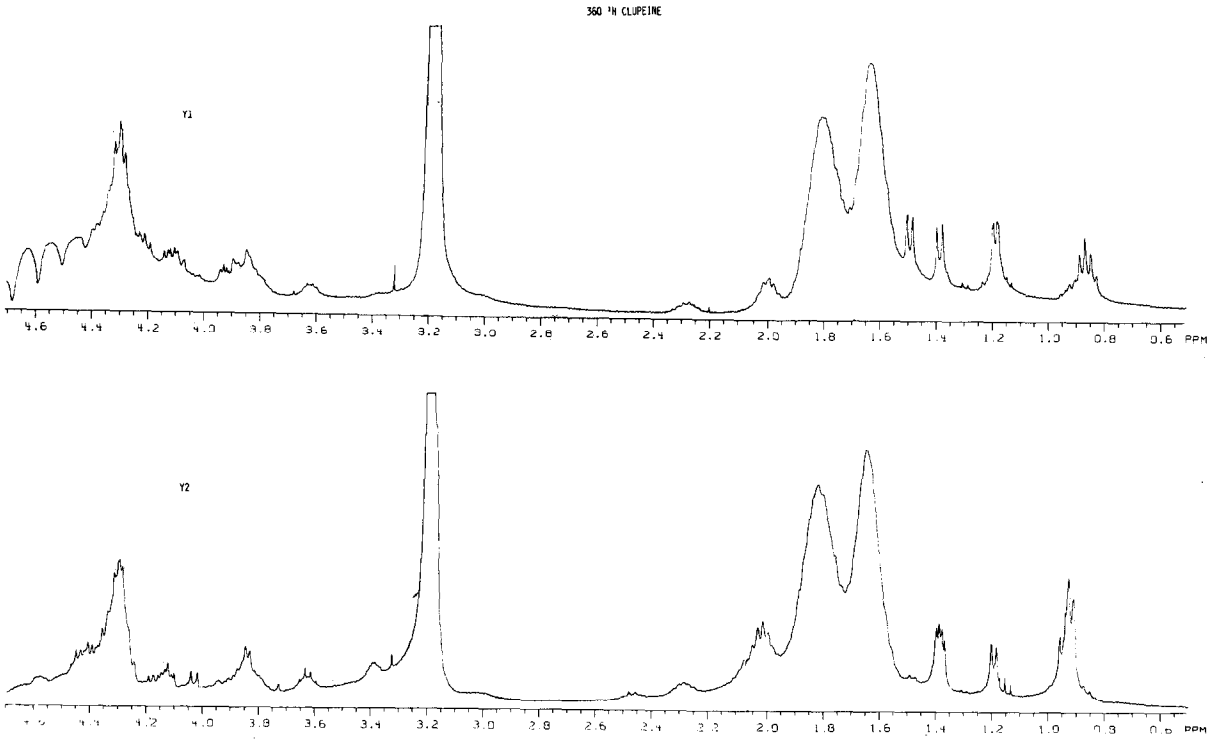

Fig.1. 360 MHz proton spectra of clupeines YI and YII at pH 9.0, 1 mM in D_2O, 27°C, 200 scans.

Table 1
Chemical shifts of assigned ^1H resonances in clupeines

	Y1					Y2					Z			
Peak	α	β	γ	δ	Peak	α	β	γ	δ	Peak	α	β	γ	δ
Ala [1]	4.10	1.50								Ala[1]	4.10	1.50		
					Ala[8]	4.27	1.34			Ala[9]	4.27	1.36		
Ala[26]	4.25	1.39			Ala[26]	4.33	1.39			Ala[27]	4.30	1.38		
Arg	~4.3	1.85	1.63	3.2	Arg	~4.3	1.85	1.63	3.2	Arg	~4.3	1.85	1.63	3.2
Gly[27]	3.92													
Ile[11]	4.20	1.85	0.90,1.2	0.86										
			(CH_3) (CH_2)	(CH_3)										
					Pro[1]	4.11	2.42 2.10	2.0	3.37					
Pro[12,18]	~3.8	2.27 2.00	2.0 2.0	3.40	Pro[11,17]	~3.8	2.27 2.00	2.0	3.37	Pro[12,18]	3.8	2.27 2.00	2.0 2.0	3.4
Ser[6,7,8]	~4.3	3.85			Ser[9,21]	~4.39	3.83			Ser[6,10,22]	~4.4	3.85		
Thr[20,21]	4.4	4.15	1.2		Thr[5]	4.42	4.16	1.19						
					Val[12,20]	4.04	2.01	0.92 0.95		Val[13,21]	4.03	1.98	0.90 0.94	

Data referenced to external TMS for clupeines, at pH 6.4, 1 mM in D_2O, 27°C, 200 scans

the upper doublet Ala 8 between Arg 7 and Ser 9. The shifts of the α-CH resonances correspond to those of α-CH of the amino acids in peptide linkage [5]. The findings in themselves are significant in that they indicate the absence of ordered structure [6]. Even given the extensive overlap of arginine lines it is not likely that a local organization exists, since the width of the arginine envelopes is temperature independent. Any local structure affecting the proline residues, would have been reflected in changes of the proline coupling constants, which are not observed. The assignments are summarized in table 1. The spectra of clupeine Z do not provide any evidence for the existence of a secondary structure but cannot be analyzed in details because of the presence of threonine-containing impurities.

A plot of the chemical shift of the α-CH resonances of Ala 1 in YI and Pro 1 in YII as a function of pH at pH > 7 is shown in fig.2. The pK values are 9.8 and 10.1, respectively, very close to those measured in the free amino acids [7], again indicating the absence of structure or significant salt-bridge interactions of the type found for instance in muscle-calcium binding parvalbumins [8]. The remainder of the spectrum shows no dependence on pH indicating that the random coil structure persists even to very high pH values (pH 11.8). Presumed helix formation at pH > 13 (= pK of arginine) is accompanied by precipitation. The ^{1}H NMR spectra of clupeines also show no significant temperature dependence, as commonly observed for random coils [6,9].

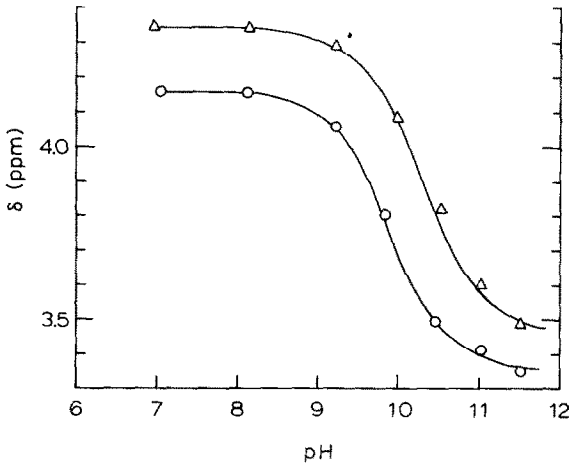

Fig.2. Titration of the N-terminal Ala (o——o) in YI and the N-terminal Pro (△——△) in YII.

Of greatest interest to us was the possibility of inducing structure by the binding of clupeines to nucleic acids or nucleic acid derivatives. The search for such an effect, however, has proved generally disappointing. Phosphates produce no significant changes in the NMR spectra of clupeines over a wide range of pH and ionic strength. Nor do clupeines produce any changes in the characteristics of the ^{31}P resonances of phosphate buffers. No effects indicating the formation of structure are observed with sulfate or perchlorate ions. The ^{1}H spectra of TMP or dGMP and clupeines are additive over a wide range of relative concentrations and nucleotide ^{31}P resonances are not affected. Similar results are obtained with dinucleotides ApA and UpA and the short double-helical strands of A_8T_8, indicating the absence of any significant structural change, although broadening of Arg resonances is seen at high A_8T_8 concentrations. Longer double-helical strands of calf-thymus DNA (300 basepairs or longer) obviously form a complex with the 3 clupeines. However, the complex immediately precipitates out of aqueous solution and its structural features cannot be observed by NMR.

4. Discussion

The findings obtained here confirm the conclusion in [2,3] that clupeines exist in random coil forms in aqueous solutions. If a structure such as that proposed in [10] does exist, it requires the absence of water or a solid matrix or both for its formation. It is not stable in aqueous solution. In addition, the data collected on this system indicate that the random coils are not readily perturbed in their average conformations by weak ionic interactions. The observed CD spectral changes on addition of inorganic ions are more likely to reflect direct electric field effects than structural alterations [2]. A minimal length of double-stranded deoxynucleotides exceeding 8 base pairs is clearly required for complex formation, and complex formation is accompanied by precipitation. The intriguing possibility remains that, at very low clupeine concentrations, complexes with long strands of DNA would remain in solution and could be observed. The concentration ratios at which such experiments are likely to be successful are not within the sensitivity limits of existing spectrometers, but they deserve to be borne in mind for the future.

Acknowledgements

The authors were assisted by Anthony Ribeiro, Michael Hogan and Vladimir Basus. The work at Stanford was supported by NIH grant RR00711 and NSF grant GP23633.

References

[1] Ando, T., Yamasaki, M. and Suzuki, K. (1973) Protamines, Springer-Verlag, Berlin.
[2] Toniolo, C., Bonora, G. M., Marchiori, F., Borin, F. and Filippi, B. (1979) Biochim. Biophys. Acta 576, 429–439.
[3] Bonora, G. M., Ferrara, L., Paolillo, L., Toniolo, C. and Trivellone, E. (1979) Eur. J. Biochem. 93, 13–21.
[4] Bray, R. P., Wade-Jardetzky, N. G., Jardetzky, O., Geisler, N. and Weber, K. (1979) J. Mol. Biol. 128, 259–264.
[5] Nakamura, A. and Jardetzky, O. (1967) Proc. Natl. Acad. Sci. USA 58, 2212–2219.
[6] Roberts, G. C. K. and Jardetzky, O. (1969) Adv. Protein Chem. 24, 447–545.
[7] Takeda, M. and Jardetzky, O. (1957) Chem. Phys. 26, 1346–1347.
[8] Nelson, D. J., Opella, S. J. and Jardetzky, O. (1976) Biochemistry 15, 5552–5560.
[9] Markley, J. L., Meadows, D. H. and Jardetzky, O. (1967) J. Mol. Biol. 27, 25–40.
[10] Ottensmyer, F. P., Whiting, R. F. and Korn, A. P. (1975) Proc. Natl. Acad. Sci. USA 72, 4953–4955.