Protamines. VII. Circular Dichroism Study of Salmine A I*

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Salmine A I, one of the components of the protamine from salmon, has been purified and characterized. The conformational preferences of salmine A I have been examined as a function of pH, added salts, presence of helix-supporting solvents, and temperature, using circular dichroism. It has been found that this small basic protein adopts predominantly an unordered conformation in aqueous solution. Addition of counter-ions, in particular perchlorate, and 2-chloroethanol induces to various extents the onset of the right-handed α-helical conformation. The results are discussed in comparison with those previously reported on the three main components of clupeine, the protamine from herring, and with the published conformational predictions by various statistical methods.

Introduction

Protamines are proteins of low molecular weight, found tightly associated with DNA in fish spermatozoa [2]. Nearly two-thirds of the amino acid residues in protamines are basic, in general arginines, and these basic residues usually occur as clusters of two to seven in a sequence [3].

Recently, we described the purification and characterization [4], and the conformational properties of the three main components YI, YII, and Z of clupeine, the protamine from herring, under various experimental conditions using CD [4], and ^{13}C and ^{1}H NMRs [5, 6]. In this paper the purification and characterization of salmine AI, one of the components of the protamine from salmon, is reported. The conformational preferences of salmine AI have been examined as a function of pH, added salts, presence of helix-supporting solvents, and temperature, using CD. The results are discussed in comparison with those previously reported of clupeines [4], and with the published conformational predictions by various statistical methods [3].

Experimental Section

Materials

Salmine sulfate (grade X) was purchased from Sigma. Microgranular pre-swollen carboxymethyl cellulose CM-52 was obtained from Whatman, carboxymethyl Sephadex C-25 from Pharmacia, and Amberlite CG-50 from Serva. 2-Chloroethanol (Fluka) was distilled and the central fractions (b. p. 128 °C at 1 atm) collected.

Methods

Amino acid analyses were performed according to standard procedures [7] on a C. Erba amino acid analyzer model 3A 28. Acid hydrolyses were carried out in 6 M HCl in evacuated tubes sealed under vacuum for 24 h at 110 °C.

CD spectra were obtained from a Cary 61 dichrograph. Cylindrical, fused quartz cells (width 0.5 mm, 1.0 mm, and 1.0 cm path length) were used. Dry pre-purified nitrogen was employed to keep the instrument oxygen-free during the experiments. Temperature was controlled by means of a hollow-walled, brass cell holder through which water was circulated. The temperature in the cell was determined using a Philips thermistor. No thermal degradation of the samples occurred, as shown by regeneration of the original spectra upon cooling. In the spectra the mean residue ellipticity values $[\Theta]$ are reported. They are defined as follows: $[\Theta] = (\Theta/10)(\bar{w}_r/l_c)$, where Θ is the measured ellipticity in degrees, l is the path length of the solution in cm, c is the concentration in g/cm^3, and $\bar{w}_r$ is the mean residue weight. The latter was taken as 206.6 for salmine AI sulfate. The CD properties were independent on concentration in every case. The CD data reported in the figures represent average values

* For part VI see ref. [1].

Abbreviations: CD, circular dichroism; NMR, nuclear magnetic resonance; ORD, optical rotatory dispersion.

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from three separate recordings. The spectra were recorded immediately after the preparation of solutions. The right-handed α-helical content of salmine A I was calculated using the relationship proposed by Greenfield and Fasman [8].

Fractionation of salmines

In a typical experiment 1.0 g of salmine sulfate, dissolved in 10 ml 0.05 M acetate buffer, pH 5.8, containing 1.5 M NaCl, was applied to a carboxymethyl Sephadex C-25 column (2.8 × 120 cm) and eluted with the same buffer. 10-ml fractions were collected. The flow rate was 100 ml/h. The elution was followed using the absorbance at 230 nm (peptide chromophore). The fractions corresponding to the salmine A I component were collected, diluted 1 : 8 with distilled water and applied to an Amberlite CG-50 column (1.6 × 10 cm). Desalting was obtained by elution with 5% acetic acid. Salmine A I, eluted with 50% acetic acid, recovered by lyophilization, redissolved in 10 ml 0.1 M H_2SO_4 in an ice-bath, was precipitated as its sulfate salt by adding absolute ethanol in excess. The homogeneity of salmine A I was controlled by analytical chromatography using a carboxymethyl cellulose CM-52 column (1.8 × 50 cm). The elution was obtained with a NaCl gradient (from 0.5 M to 1.2 M) in 0.01 M acetate buffer, pH 6.0. 5.0-ml fractions were collected. The flow rate was 30 ml/h.

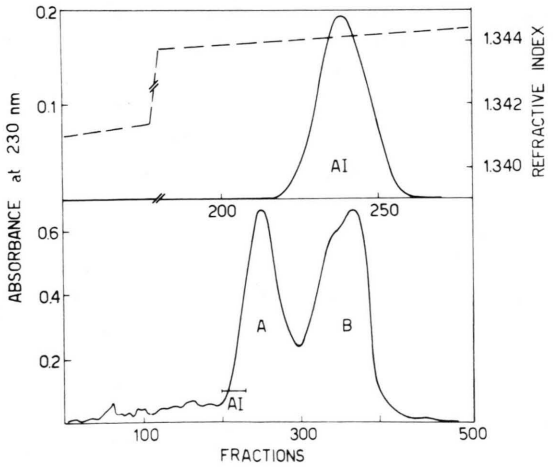

Fig. 1. (Lower part.) Purification of salmine components by chromatography on a carboxymethyl Sephadex C-25 column. The bar indicates the collected fractions. (Upper part.) Analytical chromatography of salmine A I using a carboxymethyl celluose CM-52 column.

Results and Discussion

Purification and characterization of salmine A I

The results of the column chromatographic method using carboxymethyl Sephadex C-25 for the preparative scale fractionation of salmine are illustrated in Fig. 1 (lower part). The chromatogram strictly resembles that published by Ando and Watanabe [9] using Biogel CM-2. Salmine is fractionated into two main fractions, A and B, each of them in turn consisting of two or more components [2, 9]. The homogeneity of the A I component of salmine was assessed by analytical chromatography using carboxymethyl cellulose CM-52 as described in Fig. 1 (upper part) and confirmed by amino acid analysis (Ser 3.94, Pro 3.21, Gly 2.00, Val 1.93, and Arg 20.97). These latter data fit well with those expected from the amino acid sequence of salmine A I [9]:

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1    5     10    15    20    25    30 32
PRRRRSSSRPVRRRRRPRVSRRRRRRGGRRRR
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Only traces of alanine [9], isoleucine [9], and ornithine, the hydrolysis product of arginine [10] were found in the amino acid chromatogram. The purification of the other salmine components, still contaminated after the fractionation shown in Fig. 1 (lower part), is presently in progress.

Circular dichroism study

The CD spectrum in the 250–195 nm region of salmine A I at pH 1.5 is shown in Fig. 2A. Two negative maxima at 231 nm (weak) and at 198 nm (very intense) are apparent, accompanied by a positive maximum at 218 nm of very weak intensity. The positive CD maximum at 218 nm was assigned either to the $n \rightarrow \pi^*$ or to the $\pi \rightarrow \pi^*$ transition of the peptide chromophore, while a precise conclusion is not possible as to whether the negative maximum at 231 nm is a distinct band or the long-wavelength tail of the strong band at 198 nm; this latter band should be related to the $\pi \rightarrow \pi^*$ transition of the peptide chromophore [4]. The general features of the CD curve of salmine A I are in close agreement with those already reported for the three main components of clupeine [4], and the mixture of salmines [11–13].

With increasing pH the negative maximum at 231 nm of salmine A I experiences a small blue shift with a concomitant slight increase in intensity, while

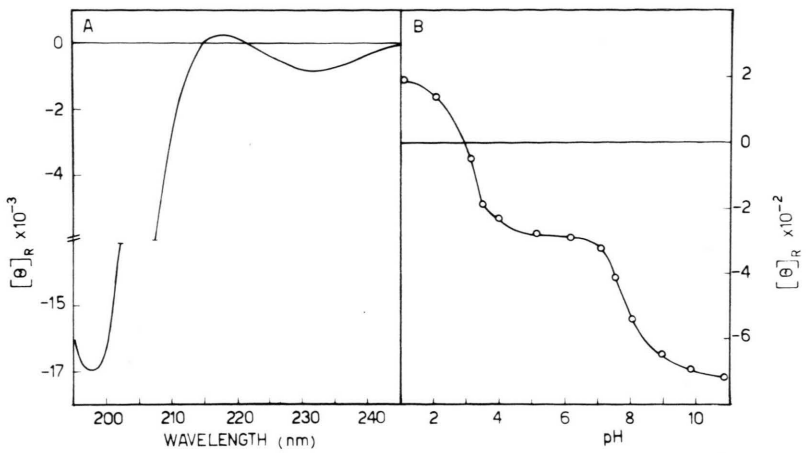

Fig. 2. A. CD spectrum in the 250–195 nm region of salmine A I in water (pH 1.5). B. Plot of residue molar ellipticities at 220 nm of salmine A I versus pH.

the positive maximum at 218 nm decreases in intensity becoming a negative minimum. Fig. 2B shows the pH dependence of the ellipticity at 220 nm for salmine AI. Two regions of pH where a change in ellipticity takes place are evident, the former (below pH 4.0) corresponding to the range of titration of the single (C-terminal) carboxyl group of the protein [9], the latter (above pH 7) corresponding to that of the single (N-terminal) secondary amino group [9]. The cross-over point is observed at pH 2.9. The close similarity of pH dependence of the CD signal of salmine A I and clupeine Y II [4] is probably associated with the presence of identical amino acid residues at the N- and C-terminal ends of their sequences.

The CD results described above strongly support the view that salmine A I, in analogy with the three main components of clupeine [4], exists as a statistical coil in water, the alteration observed in its CD spectrum as a function of pH simply reflecting a perturbation in the distribution of conformational states of a essentially unordered polypeptide chain.

The possible existence of salmine A I in the α-helical form in aqueous and/or organic solutions was examined by recording the CD spectra in sodium chloride, sodium perchlorate, and 2-chloroethanol solutions. The biological relevance of this study is based on the fact that it tends to mimic environmental conditions for salmine A I where charge repulsion from neighbouring positive side chains are minimized, *e.g.* when complexed with DNA.

Fig. 3 shows the CD curves of salmine A I in 1 M Cl^- and ClO_4^-, and in the absence of added salt at pH 6.3. The CD spectrum in 1 M ClO_4^- is reminiscent of that of the right-handed α-helical conformation [14]; however, the helicity is not higher than 10% [8]. In any case, the preferential stabilization of the perchlorate counter-ion stands out clearly. A much higher level of α-helix was induced in salmine A I by 2-chloroethanol (Fig. 4A). From the relationship introduced by Greenfield and Fasman [8] 41.4% of helicity was calculated. The present data in 1 M Cl^- and 2-chloroethanol solutions obtained by

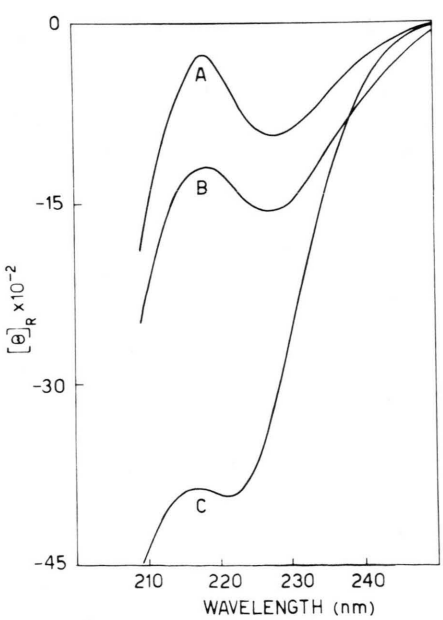

Fig. 3. CD spectra in the 250–205 nm region of salmine A I at pH 6.3 in pure water (A), 1 M Cl^- (B), and 1 M ClO_4^- (C).

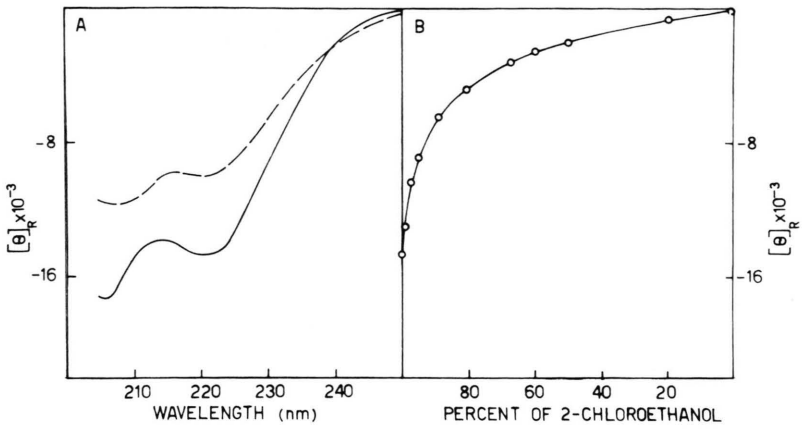

Fig. 4. A. CD spectra in the 250–205 nm region of salmine AI in 2-chloroethanol at 25 °C (——) and at 75 °C (— —). B. Plot of residue molar ellipticities at 222 nm of salmine AI versus percent of 2-chloroethanol in water (v/v).

CD measurements on salmine AI are in agreement with those previously obtained by ORD measurements on mixtures of salmines [11, 15]. Also, the results shown in Figs. 3 and 4A closely parallel those already described for the three main components of clupeine [4].

The stability of the helical structure of salmine AI in 2-chloroethanol was first determined by measuring the CD spectra in the 25–75 °C range. No dramatic alterations was noted in the CD profile (Fig. 4A); in particular, the ellipticity values at 222 nm and 207 nm (negative maxima) change only gradually. From 25 °C to 75 °C the decrease in helicity is about 35%. Further information was obtained by examining the CD properties of salmine AI in water-chloroethanol mixtures (Fig. 4B). Addition of water to the alcoholic solution of salmine AI results in an α-helix → statistical coil transition which is 50% completed at about 10% water content. Both the temperature- and solvent-directed conformational transitions are fully reversible processes. Again, the present results on salmine AI are in line with those previously reported on the clupeines [4].

One of us (C.T.) recently determined [3] the secondary structures of thirteen fish protamines by the statistical method of Chou and Fasman [16] as well as by two modifications of it [3, 17]. Concerning salmine AI, the original Chou-Fasman algorithm [16] predicted correctly the absence of helical structure in aqueous solution. In contrast, even the modification proposed by Toniolo [3] and that introduced by Dufton and Hider [17], which predicted rather accurately the occurrence of helical regions in clupeines in structure-supporting organic solvents, that is their maximal helical potential, failed to predict helical regions in salmine AI. Interestingly, however, the hexapeptide segments of salmine AI starting with residues 11 and 18 to 21 have helical potentials (N_α) not much lower the cut-off value ($N_\alpha = 1.0$) (Fig. 5). The two segments sum up 15 amino acid residues which correspond closely to the helical percent (41.4%) found in 2-chloroethanol. The helix-breaking points are to be found in the N-terminal part in the Ser^6-Ser^7-Ser^8 sequence and in the Pro^{10} residue, in the central part in the Pro^{17} residue, and in the C-terminal part in the Gly^{27}-Gly^{28} sequence.

Finally, since native protamines interact strongly with DNA [2], it seemed of interest to study the CD properties of salmine AI as a function of phosphate ions. At pH 9.0 the CD spectrum does not seem to be sensitive to the addition of HPO_4^{2-} ions, at least to 0.4 M concentration. This finding is in accord with that already reported for clupeine YII, which also has a proline residue at its N-terminus [4].

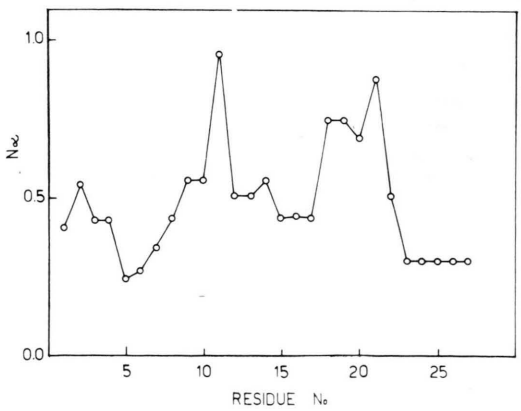

Fig. 5. Prediction of α-helical regions in salmine AI according to the Chou-Fasman method [16] as modified by Dufton and Hider [17].

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