AVIAN ERYTHROCYTE HISTONES: SALT-INDUCED CONFORMATIONAL CHANGES IN THE ERYTHROCYTE-SPECIFIC HISTONE V (f2c)*

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1. Introduction

Eukaryotic chromosomal DNA consists mainly of deoxyribonucleic acid (DNA) and two predominant classes of proteins, histones and nonhistones [1]. The histones constitute the larger group and two biological roles have been suggested for them: the first is to promote structure and packaging of the genetic apparatus, the chromosome; the second is to provide a general repression of nonspecific genetic information within the DNA.

One of the more interesting of these histones is V or f2c [1, 2] which is found exclusively in the avian erythrocyte where it is believed to be synthesized and accumulated during the terminal stages of erythrocyte differentiation [3–6]. It has been suggested that V possesses both the histone biological roles, in that it not only provides for the unique structural features of the mature erythrocyte interphase chromatin [4, 7–9], but also aids in the near complete repression of the DNA [9, 10].

Conformational changes occurring in this histone upon binding to DNA or as influenced by changes in the nucleoplasmic environment could provide one of the mechanisms by which it executes both of these predescribed functions. Presumably in the cell, post-synthetic modifications (phosphorylation and acetylation) [4, 11–13] may be of major importance. According to NMR studies of V in D_2O and 1.0 M $NaCl/D_2O$, this histone undergoes a reversible conformational change [14]. In this report we present evidence obtained from circular dichroism studies of V that a conformational change occurs upon changing the ionic environment of this histone, that this change occurs at ionic strengths within the physiological region, 0.1–0.2 M, and that a substantial proportion of the resulting form is one which resembles a standard β-structure.

2. Materials and methods

Histone V was obtained from isolated mature goose erythrocyte nuclei by selective acid extraction using 10 volumes of 0.15 MNaCl, pH adjusted to 1.85 with conc. HCl at 4°C and further purified by ion exchange [2, 13] and exclusion chromatography [11, 15]. The purity of this fraction was assessed by electrophoresis in acidic (pH 2.7 [16]) and SDS [17] polyacrylamide gels.

All circular dichroism measurements were made on a Cary 61 spectropolarimeter at room temp. in 0.05 cm quartz cells. The salt concentration of histone in 0.001 M Tris-HCl, pH 7.0 (ca. 1 mg/ml) was progressively changed by additions of small aliquots of a concentrated aqueous solution of the salt under investigation. Histone concentration was estimated by weight and by turbidiometric measurements. The mean residue molecular weight used (107) was calculated from the amino acid composition of this histone [15]. The data presented in figs. 2–4 are expressed in terms of $[\theta]$, the specific ellipticity, deg-cm^2/decimole.

* N.R.C.C. No. 13232.

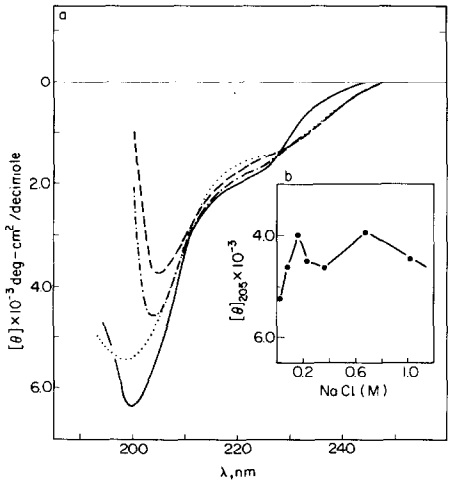

Fig. 1. The effects of sodium chloride on the CD spectrum of histone V. All solutions contained Tris-HCl, 0.01 M, pH 7.0. (——) Tris-HCl; (· · · · · · ·) 0.078 M NaCl; (- - - - - - -) 0.15 M NaCl; (-·-·-·-) 0.66 M NaCl. Inset: Variation of ellipticity at 205 nm with salt concentration.

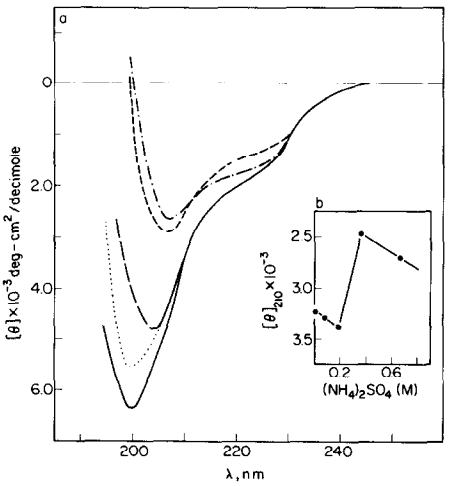

Fig. 2. The effects of ammonium sulfate on the CD spectrum of histone V. All solutions contained Tris-HCl, 0.01 M, pH 7.0. (——) Buffer; (· · · · · · ·) 0.078 M; (— — —) 0.19 M; (-·-·-·-) 0.36 M; (- - - - - - -) 0.67 M. Inset: Variation of ellipticity at 210 nm with salt concentration.

3. Results and discussion

Circular dichroic spectra have proven useful in the estimation of the overall conformation of many proteins [18, 19]. When the spectra of several proteins were examined and compared with the "standard" or "library" spectra for α-helical, β-structure and random coil conformations good agreements between measured and computed spectra were observed [20]. Some measure of agreement was also noted between the calculated amount of each of the three conformations and that seen in the X-ray crystallographic structure of the same proteins. The circular dichroic spectrum of histone V was examined to determine if it existed in any one or a mixture of the "standard" conformations.

The CD spectrum of histone V in aqueous solution (fig. 1) indicates that the protein contains a large proportion of the random coil conformation ($[\theta]_{190\,nm}$ = 6,250). This CD spectrum is similar to that produced by a dry unoriented film of poly L-lysine at pH 7.8 [21, 22]. The restricted motion in the dried film presumably prevents the generation of long stretches of the "organized random coil" form normally observed in solution and may be more representative of the random coil form of proteins in solution. Histone V also contains a substantial amount of some other conformation resembling β-structure ($[\theta]_{220\,nm}$ = 2,000). Evidence that this histone contains some β-structure, as expected from its amino acid composition, has been obtained from an examination of solution and solid-phase infrared spectra [23].

The influence of ionic environment upon the "random coil" form of V was investigated by progressively increasing the amount of salt in the solution. Protein conformation is known to be affected by different salts: NaCl and $(NH_4)_2SO_4$, support α-helix formation in collagen and ribonuclease solutions [24], while $CaCl_2$ inhibited α-helix formation under the same experimental conditions. The results of increasing salt concentrations with each of these three salts are presented in figs. 1, 2 and 3. With each salt an abrupt structural transition occurs between 0.1 and 0.3 M and the transition molarity depends on the salt used. The greatest changes in ellipticity in going from the "random coil" form to the "physiological salt" form (i.e. the form in 0.1–0.2 M NaCl) occur in the π–π^* region of the spectrum, 190–220 nm. In spite of the changes in the ionic environment only minor changes are observed in the η–π^* region of the spectrum, 220–230 nm. The resultant "physio-

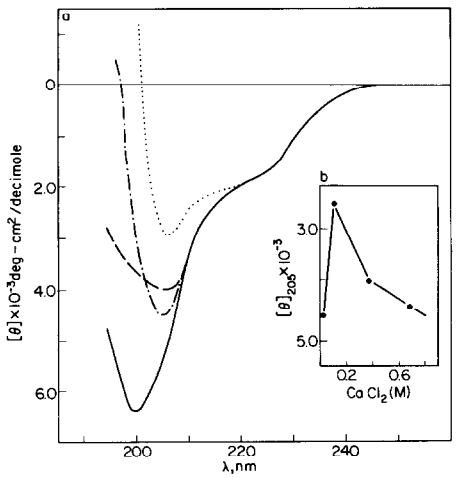

Fig. 3. The effects of calcium chloride on the CD spectrum of histone V. All solutions contained Tris-HCl, 0.01 M, pH 7.0. (——) Buffer; (·····) 0.095 M; (—·—·—) 0.36 M; (— — —) 0.67 M. Inset: Variation of ellipticity at 205 nm with salt concentration.

logical salt" conformation appears to be a mixture of the various "standard" conformations and resembles in band shape and position the spectra that have been observed for whole histone and histones f2a2 [23, 25, 26]. However, a conformation resembling the β-structure in CD properties must contribute substantially to the overall conformation. The invariant nature of the η–π^* region also implies that little change in this structure occurs with increases in the salt concentration.

Increasing NaCl concentrations beyond 0.3 M causes further conformational changes. The high-salt form observed at 0.66 M may be very similar to that generated in 1.0 M NaCl and observed in the NMR experiments. However, concentration differences (1 mg/ml for CD experiments *vs.* 10–50 mg/ml for the NMR experiments) and the possibility of different states of aggregation have not been ruled out so that the conformation obtained from the NMR experiments [14] may be different from that observed here. In $(NH_4)_2SO_4$ solutions at the higher molarities (fig. 2) only minor changes are observed in the spectrum of the histone, thus indicating preferential stabilization of the conformation of the histone. With $CaCl_2$ structural destabilization seems to be occurring at the higher salt concentrations as evidenced by the loss of ellipticity in the 200 nm region of the spectrum (fig. 3). These large disruptions with $CaCl_2$ parallel this salt's behaviour in the collagen and ribonuclease systems [24]. The small variation in the ellipticity in the 220–230 nm region again indicates that a major portion of the structure is not influenced by changes in the ionic environment.

The intermediate conformational state occurring at approximately physiological salt concentration (0.15 M NaCl) could represent the conformational state which binds most effectively with DNA and accounts for the near-total repression of the template activity of the erythrocyte chromatin [4, 9, 10]. It is interesting to note that the amount of chromatin-DNA made available for transcription *in vitro* and ethidium bromide primary binding [9, 10] after selective removal of V from mature erythrocyte chromatin closely parallels the amount of V estimated to be present per nucleus [1]. Recent studies on the complexes between V and DNA (0.15 M NaCl, pH 8) have indicated that no disruption of the DNA helix geometry had occurred upon the complex formation [27]. The observed physiological salt conformation in this study may also be the form responsible for these observations.

The conformational changes in V, i.e. from a "random" to that defined by physiological salt conditions could be related to the observed influence of salt upon native nuclei [7] and may reflect, in part, the structural role played by this histone. Other environmental variables could alter the conformation of V. Conceivably, insipient post-synthetic chemical modification such as acetylation [12, 13] and phosphorylation [4, 11] would generate a weaker interaction with chromatin-DNA, thus accounting for the recent observations by Gasavyan and Andreeva [28] who showed by selective extraction of V from erythroblast chromatin that V does not effect template activity as much as in mature erythrocyte chromatin [4, 9, 10]. In this respect, erythroblast chromatin is similar to that of liver chromatin [29]. However, it should be noted that erythroblast chromatin is obtained from a mixture of immature cell types and amounts of immature cell types which do not contain V must be considered. Experiments are now underway to elucidate in detail the specific conformation adopted by V both in solution and in chromatin.

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