SALT AND TEMPERATURE INDUCED CONFORMATIONAL CHANGES OF PHOSPHATE-RICH AND PHOSPHATE-DEPLETED AVIAN ERYTHROCYTE SPECIFIC HISTONE V (f2c)

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Received 9 April 1975

1. Introduction

Recently it was shown that phosphorylation and dephosphorylation of V both occur following its synthesis [1]. This study utilized and attenuated linear salt gradient with cation-exchange chromatography to separate significant amounts of newly synthesized, phosphorylated histone V from older, largely dephosphorylated histone V. In the present report the circular dichroic (CD) properties of two such types of V were examined in basically the same manner described earlier [2] in order to determine if quantitative differences in covalently bound PO_4 can alter histone V conformation. The CD spectral results indicate that the amount of phosphorylation of histone V has several effects on the overall histone structure so as to modify its conformation in relation to changes in environment such as ionic strength and temperature.

2. Materials and methods

Histone V was obtained from nuclei isolated from immature blood cells fractionated by isopycnic centrifugation [3]. The method for the fractionation of histone V has been described [1]. For this study the elution profile was divided into 5 fractions and fraction 1 and 4 were used. Protein from both fractions were identical in molecular weight according to gel electrophoresis [1] and amino acid composition but differed considerably in bound phosphate (6.48 and 0.96 μg PO_4/mg protein, respectively.

All CD measurements were made on a calibrated Cary-Varian spectropolarimeter at 30 ± 0.2°C in 0.05 cm and 1.0 cm thermostatted quartz cells [4]. Initial estimates of the histone concentration were made by turbidometric measurements [8] on freshly prepared solutions of lyophilized histone. These estimates are 1.9 times higher than those determined by amino acid analysis of solution aliquots. For this study all histone concentrations were determined by amino acid analysis [5] of an aliquot of the solution used for measurement. Mean residue concentrations were calculated from the amino acid analysis data assuming a lysine content of 24%, a protein mol. wt of 18 500 [1] and a mean residue mol. wt of 107. The salt concentration of the histone solution in 0.01 M Tris–HCl, pH 7.5 (1 mg/ml) was changed in the manner previously described [2]. The data presented are expressed in terms of $\epsilon_L - \epsilon_R$ (M^{-1} cm^{-1}), the molar dichroic absorption, and are based on the mean residue molecular weight concentration.

3. Results and discussion

Both the high phosphate fraction 1 and the low phosphate fraction 4 have similar CD spectra insofar as the positions of the observable bands are concerned (fig.1a). Each fraction possesses three main bands. The first, centered at 198 nm, is presumed to be connected with π–π^* amide bond transitions and is indicative of the extent of 'random' structure found in the peptide backbone of proteins [6–8]. The second, centered at 222 nm, is presumed to be connected with n–π^* amide bond transitions and indicates the extent of β-like

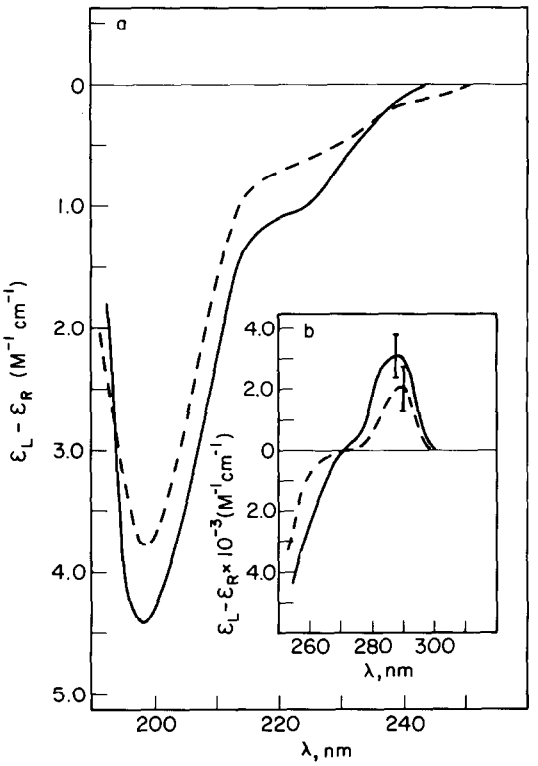

Fig.1.a.) The circular dichroism spectrum of histone V fractions 1 (——) and 4 (-------). Solution: pH 7.5, Tris–HCl, 0.01 M. Temperature: 27.0 ± 0.2°C. Mean residue molecular weight concentration: 2.5–4.5 × 10^{-3} M. b) Conditions: as described for 1a. Vertical bars indicate the errors associated with measurements.

structures found in the peptide backbone of proteins. The third, a weak band centered at 288 nm (fig.1b), is assumed to be connected with π–π^* aromatic ring transitions and indicates the extent of order involved in the aromatic chromatophores found in the protein, e.g. those of phenylalanine and tyrosine [9]. The CD spectra of the two fractions differ with respect to the intensity of each of these bands; fraction 1 has more ellipticity associated with all three transitions. This suggests that fraction 1 has more random structure, more β-like structure and is more structurally ordered in the aromatic amino acid region of the protein than fraction 4.

Changes in the ionic strength of the solvent can induce conformational changes in histone V [2]. These changes were also dependent, to a certain degree, on the type of salt used, i.e. either on structure making salts like NaCl and $(NH_4)_2SO_4$ or on a structure breaking salt like $CaCl_2$ [10]. Repetition of this experiment using the two histone fractions indicated that some similarities and differences between these two fractions occur. Both fractions appeared to undergo conformational changes in the randomly oriented regions (200 nm) with changes in salt concentration (fig.2a–c). Only very minor changes (<5%) were observed in the β-like areas (at 222 nm). With increasing concentrations of the structure making salts both histone fractions appeared to adopt a distinct final conformation that was dependent not only on the histone fraction but also on the salt concentration (fig.2). With increasing amounts of the structure breaking salt, $CaCl_2$, only fraction 4 adopted a distinct conformation (fig.2) albeit slightly different from that in the structure-making salts. Fraction 1 in this salt underwent a progressive conformational change but did not adopt a distinct final conformation at the salt concentrations investigated. Both histone fractions responded differently to changes in the concentrations of salt (fig.2). In all salts fraction 1 was partially converted to its final conformational form when fraction 4 was fully converted. It may be noted that the NaCl concentration where fraction 4 was fully converted to its final form was approx. 0.15 M, a behaviour not unlike that of the whole histone V from mature erythrocyte nuclei [2] where little or no phosphorylation activity occurs [5].

It was reported that low temperatures altered the electrophoretic mobility of histone V [11]. These changes in mobility might be partially related to changes in conformation of the histone with temperature. We examined the conformational changes that occur upon lowering the temperature to 3.0°C. At low salt concentrations no temperature dependence of the CD spectrum was observed. At high salt concentrations (>0.2 M) a temperature dependence of the randomly oriented regions of each of the histone fractions was observed (fig.2a–c). With NaCl both histone fractions behave similarly to the change in temperature and indicated that by the shift of the ellipticity towards the low salt form the formation of more random structure in the protein was occurring upon lowering the temperature. In $CaCl_2$ and $(NH_4)_2SO_4$ solutions the two fractions behaved differently from each other. Fraction 4 seemed to be the least perturbed by changes in temperature, the response being approximately the

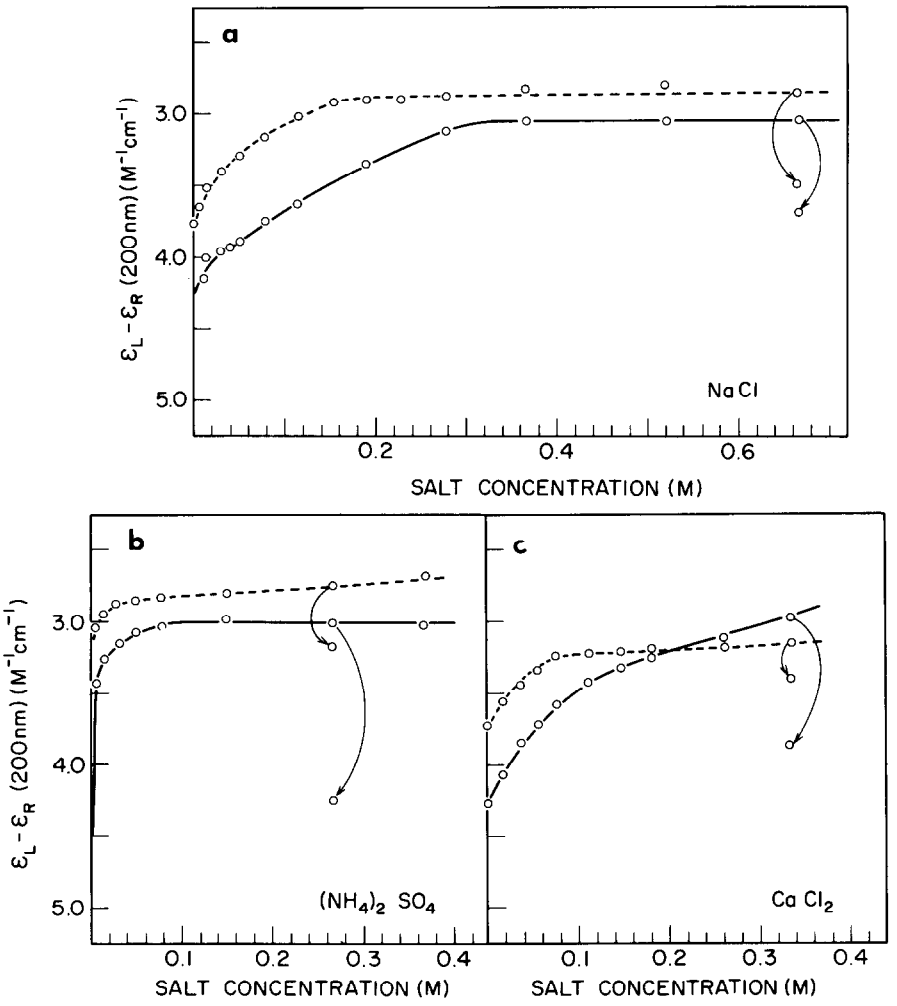

Fig.2. Variation of the molar dichroic absorption $\epsilon_L - \epsilon_R$, of histone V fractions 1 (———) and 4 (– – –) with sodium chloride (a), ammonium sulfate (b) and $CaCl_2$ (c). Conditions: as described in fig.1. The points indicated by the arrows show the shift in dichroic absorption which occurred upon lowering the solution temperature to 3°C.

same as had been observed in the NaCl solutions. Fraction 1 underwent a much larger conformational change and returned almost fully to its low salt conformation.

Phosphorylated V may not bind the erythrocyte chromatin as tightly as dephosphorylated V [1,12,13]. This observation holds true either for mature or immature erythrocyte populations and appears to be closely related to the concentration and metabolic properties of 'newly' synthesized histone V [1]. These results suggest newly phosphorylated V binds weakly to chromatin or it is located in nuclear compartments more amenable to extraction than the lesser phosphorylated, 'older' molecules of V. Our conformational studies ([1] and figs. 1 and 2) as well as a similar report [13] in which mature and reticulocyte-enriched whole chicken erythrocyte V was compared, provide evidence for histone V conformational changes mediated by phosphorylation. Although we know fraction 1 is greatly enriched in 'newly' synthesized and phosphorylated histone in comparison to fraction 4 [1], the fractionation technique used still

gives an admixture of molecules which we believe range from possibly several phosphorylation sites [5] to no sites, but whose average would be at least PO_4 residue per molecule of V for fraction 1 and about 1/10th this value for fraction 4. Nevertheless, it represents an appreciable difference in comparison to whole extracts [12]. However, the absolute number of modification sites and their location within the primary sequence is unknown. Recent reports on the N-terminal sequence and the sequence adjacent to the only phenylalanyl residue of chick histone V indicate three tyrosyl residues [14] and the phenylalanyl residue [15] are within sequences containing seryl and threonyl residues, potential sites of modification [5].

Near or proximate modification sites could account for the effects observed in the region of the aromatic chromophores (fig.1b). These effects might arise from changes in the structural organization within these regions brought on by electrostatic repulsions or by protein-protein interactions. If similar capabilities occur in vivo these conformational differences may serve to diminish histone interaction or binding to chromatin subcomponents (protein and/or nucleic acids) thus giving rise to some of the observed differences in fibrillar structure [12,16,17], template and metabolic properties [1,3,5] of chromatin from erythroblast and erythrocyte enriched cell populations. However, modification of histone V could also serve to enhance chromatin binding specifically and this alternative explanation should be tested.

Acknowledgements

We thank Michael Dove for technical assistance and Alex Castagne for the amino acid analysis. V.L.S. expresses his gratitude to Dr Michael Sung, University of Southern Illinois for earlier discussions and for a copy of his manuscript [13].

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