Conformational Changes of Histone LAK (f2a2)†

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ABSTRACT: Conformational changes in histone LAK (IIb1; f2a2) have been followed by measuring the fluorescence anisotropy of the tyrosine emission and the circular dichroism (CD). These were examined as functions of salt concentration and pH. Addition of sodium phosphate induces instantaneous changes as seen by our techniques, but sodium chloride induces small amounts of slow change in the fluorescence properties. Although histone LAK responds to smaller concentrations of phosphate than of chloride, chloride induces a greater α-helical content (22 *vs.* 17). $MgCl_2$ and NaCl induce the same changes in CD. Histone LAK is more sensitive to salt and pH changes than either histone GRK (IV; f2a1) [Wickett, R. R., Li, H. J., and Isenberg, I. (1972), *Biochemistry 11*, 2952] or histone KAS (IIb2; f2b) [D'Anna, J. A., Jr., and Isenberg, I. (1972), *Biochemistry 11*, 4017]. Histone LAK undergoes conformational changes corresponding to titration of the acidic groups of the protein. Changes in histone LAK do not satisfy a two-state, independent salt-binding model as do changes in histones KAS and GRK.

Histones are the most abundant of the chromosomal proteins and have been the subject of an increasing number of investigations (Bradbury *et al.*, 1965, 1967; Jirgensons and Hnilica, 1965; Taun and Bonner, 1969; Boublik *et al.*, 1970a,b; Barclay and Eason, 1972; Diggle and Peacocke, 1971; Edwards and Shooter, 1969; Li and Isenberg, 1972; Li *et al.*, 1972; Wickett and Isenberg, 1972; Wickett *et al.*, 1972; D'Anna and Isenberg, 1972, 1973).

Histone GRK[1] undergoes conformational changes in salt solutions (Li *et al.*, 1972; Wickett *et al.*, 1972) or in the presence of nucleotide triphosphates (Wickett and Isenberg, 1972). The conformational changes of histone may be decomposed into a fast change followed by slower changes which occur over a period of minutes. The fast step is characterized by increased tyrosine rigidity, α-helix formation of 16–22 residues, and some degree of histone–histone interaction which, in concentration studies, appears to be dimerization. In the slow step there is aggregation, increasing fluorescence anisotropy, and β-sheet formation (Li *et al.*, 1972; Wickett *et al.*, 1972; Small *et al.*, 1973; Smerdon and Isenberg, 1973).

In contrast to the behavior of histone GRK, histone KAS undergoes only a fast change characterized by increased tyrosine rigidity, α-helix formation of about 17 residues, and some degree of histone–histone interaction (D'Anna and Isenberg, 1972, 1973). Histones KAS and GRK interact strongly to form an equimolar complex (D'Anna and Isenberg, 1973).

We now report a study of the conformational changes of histone LAK as monitored by intrinsic tyrosine fluorescence anisotropy and by circular dichroism (CD). Measurements have been performed as functions of sodium chloride and sodium phosphate concentration at pH 7.0, and as functions of pH. The conformational changes in histone LAK differ appreciably from those in histones GRK or KAS.

Experimental Section

Calf thymus histone LAK was prepared by the general method of Sugano *et al.* (1972). Histone LAK, containing 4–6% histone ARE impurity, was treated with cyanogen bromide and chromatographed on Sephadex G-100 (4.0 × 190 cm). Selected fractions from the column were lyophilized from 0.005 M HCl. Polyacrylamide gel electrophoresis (Panyim and Chalkley, 1969) gave a single band, and the amino acid analysis gave good agreement with the published sequence (Yeoman *et al.*, 1972). Nevertheless, we have found small quantitative differences, from batch to batch of histones, in their physical properties. This variation will be discussed later.

Fluorescence anisotropy was measured at 22.0° as previously described (D'Anna and Isenberg, 1973). CD spectra were measured with a vacuum CD spectrometer built by W. C. Johnson, Jr. (Johnson, 1971), or with a Durrum-Jasco Model J-10 CD recorder. CD spectra are reported as $\Delta\epsilon = \epsilon(\text{left}) - \epsilon(\text{right})$ in units of cm^{-1} l./mol of residue.

The concentration of histone LAK was determined from measurement of the absorbance of solutions of histone LAK in water at 275.5 or 230 nm. Absorbance coefficients of 4.05×10^3 and 4.3×10^4 were used. The absorbance coefficient at 275.5 nm was assumed to be the sum of the absorbance coefficients of the aromatic residues (D'Anna and Isenberg, 1972), and the coefficient at 230 nm was calculated from the absorbance ratios.

Solutions of histone LAK were prepared by dilution of stock solutions of histones in water. Stock solutions of about 1.0×10^{-4} M were pipetted into test tubes, and salt solutions were added. The tube was closed and then inverted several times. For measurements as a function of time, the resulting solutions were transferred as quickly as possible (1–2 min) to appropriate cells and instruments.

The fluorescence anisotropy of 2.0×10^{-5} M histone LAK solutions was measured as a function of pH. Titrations were made by starting with solutions in water at pH 4.05 and titrating with 0.020–0.180 ml of NaOH or HCl. A Corning Digital pH meter, Model 112, equipped with a Corning semimicro combination electrode, was used for pH determinations. The pH of a sample was measured directly in the fluorescence cuvet while being stirred with a magnetic bar. Recorded pH represents the mean of the values before and after measure-

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[1] The nomenclature used in this paper is described in Huberman (1973): GRK = f2a1 = IV; LAK = f2a2 = IIb1; KAS = f2b = IIb2; ARE = f3 = III.

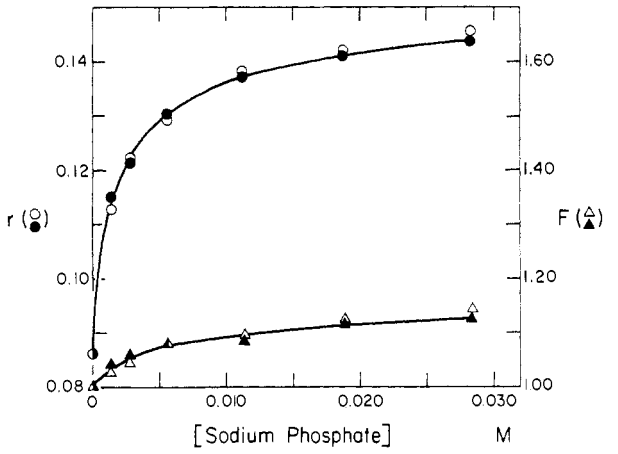

FIGURE 1: Anisotropy, r, and relative fluorescence intensity, F, of histone LAK (c = 1.0 × 10^{-5} M) as functions of sodium phosphate concentration, pH 7.0: anisotropy within a few minutes of preparation (●), and after 24 hr (○); relative fluorescence intensity within a few minutes of preparation (▲), and after 24 hr (△).

ment of the fluorescence properties. Below pH 7.0, the pH values before and after fluorescence measurements were the same; above pH 7.0, the before and after values differed by at most 0.35 pH unit.

Results

Effects of Salt and pH upon Fluorescence Emission Properties. Addition of sodium phosphate (pH 7.0) to aqueous solutions of histone LAK results in fluorescence changes which are instantaneous by our techniques. Following this, there is no further change even after 24 hr at room temperature (Figure 1). On the other hand, addition of sodium chloride to solutions of histone LAK in 0.005 M cacodylate (pH 7.0) leads to both fast and small slow changes. Although no slow change is seen in 6 hr, small, but definite changes are seen after 24 hr at room temperature (Figure 2). The magnitude of the slow change increases with sodium chloride concentration. These time dependent changes are very slow and small as compared to the

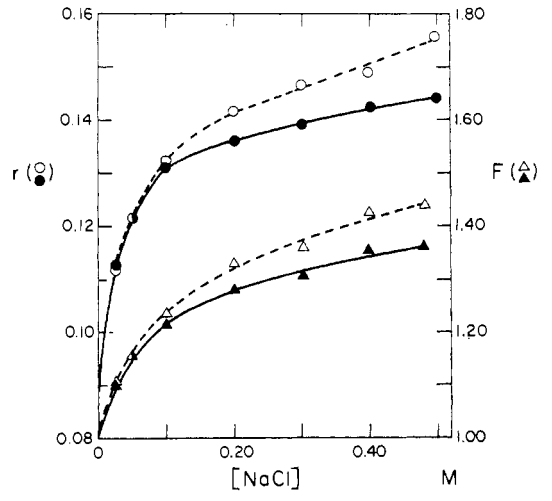

FIGURE 2: Anisotropy, r, and relative fluorescence intensity, F, of histone LAK (c = 1.0 × 10^{-5} M) as functions of sodium chloride concentration in 0.005 M cacodylate, pH 7.0: anisotropy within a few minutes of preparation (●), and after several hours (○); relative fluorescence intensity within a few minutes of preparation (▲), and after several hours (△).

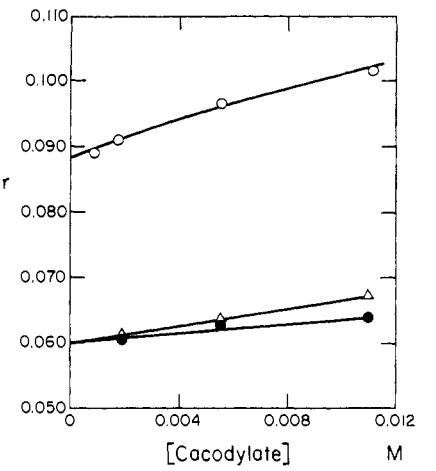

FIGURE 3: Fluorescence anisotropy of histones LAK (○), histone KAS (△), and histone GRK (●) *vs.* cacodylate concentration at pH 7.0. The histone solutions are all 1.0 × 10^{-5} M.

slow changes of histone GRK (Li *et al.*, 1972; Wickett *et al.*, 1972).

The fluorescence anisotropy is found to increase with increasing concentrations of sodium phosphate or sodium chloride in 0.005 M cacodylate (pH 7.0). However, addition of sodium chloride leads to a considerable fluorescence intensity rise, while there is only a very small intensity enhancement when phosphate is added.

In general, the fluorescence properties of histone LAK are much more sensitive to salts and pH changes than either histone KAS (D'Anna and Isenberg, 1972) or histone GRK (Li *et al.*, 1972; Wickett *et al.*, 1972). It is also more sensitive to cacodylate buffer. This is illustrated in Figure 3 which shows the dependency of the anisotropy of histones LAK, KAS, and GRK on cacodylate concentration. Cacodylate, it should be noted, had been used in previous work (Wickett *et al.*, 1972; D'Anna and Isenberg, 1972) as a convenient buffer for sodium chloride titrations because cacodylate itself had little or no effect on the CD and fluorescence properties of histones KAS or GRK. We further note that although the fluorescence anisotropy of LAK varies with cacodylate, the fluorescence intensity does not. At zero cacodylate concentration, the anisotropy of LAK is about 0.028 unit larger than that of KAS or GRK (Figure 3).

Plots of fluorescence intensity and anisotropy of histone LAK as functions of pH are given in Figure 4. It is clear that ionization of the acidic groups and, possibly, the neutraliza-

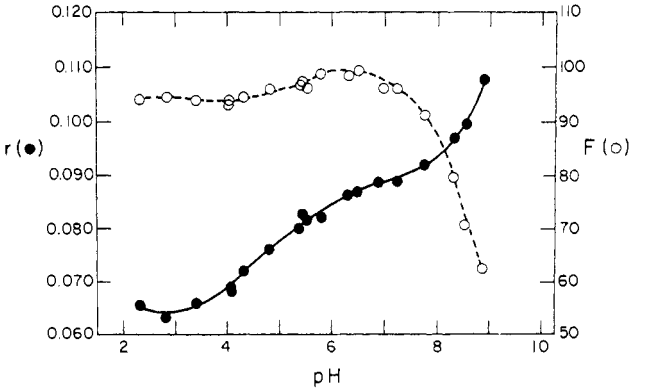

FIGURE 4: Fluorescence anisotropy (●) and relative fluorescence intensity, F (○), of histone LAK (c = 2.0 × 10^{-5} M) as functions of pH.

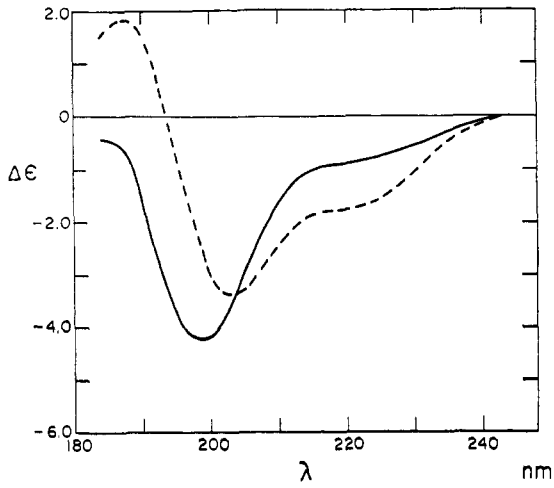

FIGURE 5: Circular dichroic spectra of histone LAK (0.58×10^{-5} M) in 0.0001 M HCl (—) and 0.0033 M phosphate, pH 7.0 (- - -), as measured on the vacuum CD spectrometer (Johnson, 1971). Samples were measured in a 1.0-mm cell.

tion of histidine induce conformational changes in histone LAK.

CD Spectra: Salt and pH Effects. Circular dichroic changes induced by both sodium chloride and sodium phosphate are instantaneous, and there is no change in the CD spectra after 24 hr at room temperature. Hence, the CD of histone LAK in sodium chloride–cacodylate does not exhibit the slow time dependence detected by anisotropy measurements. Far-uv–CD spectra of histone LAK in solutions of 1.0×10^{-4} M HCl and 0.0032 M phosphate (pH 7.0) have been measured to 190 nm using the vacuum CD spectrometer (Figure 5). CD spectra measured with the Durrum-Jasco instrument for a number of solvents are given in Figure 6a; and CD difference spectra, computed from the spectra of LAK in solutions of sodium chloride or sodium phosphate, and the spectrum at a 1.0×10^{-4} M HCl solution, are given in Figure 6b. The CD spectrum of histone LAK in 1.0×10^{-4} M HCl is similar to those obtained for histone GRK (Li *et al.*, 1972; Shih and Fasman, 1971) or histone KAS (D'Anna and Isenberg, 1972) in water, and they are typical of nonordered proteins (Tiffany and Krimm, 1969). The CD spectrum of histone LAK at 1.0×10^{-4} M HCl is essentially that for a coil, and we use the difference spectrum method (Li *et al.*, 1972; Wickett *et al.*, 1972) to estimate the nature of the conformational changes. These estimates are based on a comparison of the shape of a histone difference spectrum, computed by subtraction of the spectrum of the coil form from the spectrum of interest, with standard difference spectra. The standard difference spectra are computed from that for the histone random coil and the α helix of polylysine. The computed standard difference spectra of histone LAK are nearly superimposable on those generated for histone KAS (D'Anna and Isenberg, 1972) and need not be repeated here.

Comparison of the difference spectra of Figure 6b with the standard curves shows that the change in secondary structure, induced by salt for histone LAK, is essentially random coil to α helix for a certain number of residues. The same type of conformational change has been seen in measurements of KAS and GRK histone solutions (Li *et al.*, 1972; Wickett *et al.*, 1972; D'Anna and Isenberg, 1972).

The CD values at 220 nm of histone LAK have been plotted as a function of sodium chloride and phosphate concentration in Figure 7. The very rapid increase in the CD at very

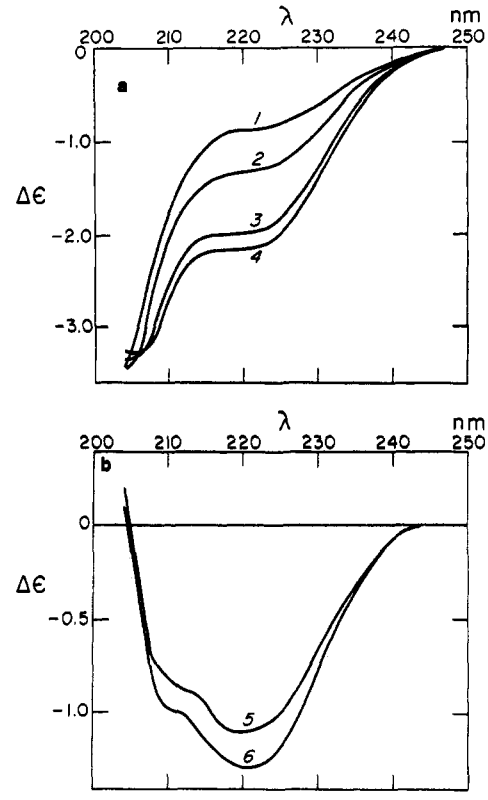

FIGURE 6: (a) CD spectra to 204 nm, of histone LAK (0.75×10^{-5} M) in 0.0001 M HCl (1); 0.002 M cacodylate (pH 7.0) (2); 0.025 M phosphate (pH 7.0) (3); 4.0 M NaCl–0.002 M cacodylate (pH 7.0) (4); samples were measured in a 2.0-mm cell on the Durrum-Jasco CD recorder. (b) CD difference spectra computed from the data in Figure 6a. Curve 5 is curve 3 minus curve 1 of Figure 6a. Curve 6 is curve 4 minus curve 1.

low salt concentrations may be noted. The CD value at zero salt, pH 7.0, has been obtained from extrapolation to zero salt of CD measurements as a function of cacodylate concentration. Although not shown in Figure 7, we note that Mg^{2+} may replace Na^{+} with no observable difference in the CD measurements. This insensitivity to magnesium ions is somewhat surprising in light of the conformational changes induced by titration of the histone acidic groups.

Analysis of Salt Effects. In previous studies (Li *et al.*, 1972; Wickett *et al.*, 1972; D'Anna and Isenberg, 1972) we found that the data followed a two-state equation derived

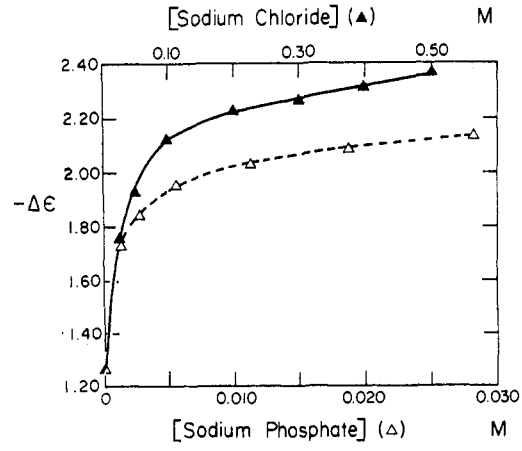

FIGURE 7: CD at 220 nm of histone LAK ($c = 1.0 \times 10^{-5}$ M) as a function of sodium phosphate, pH 7.0 (△), or of sodium chloride–0.005 M cacodylate, pH 7.0. (▲).

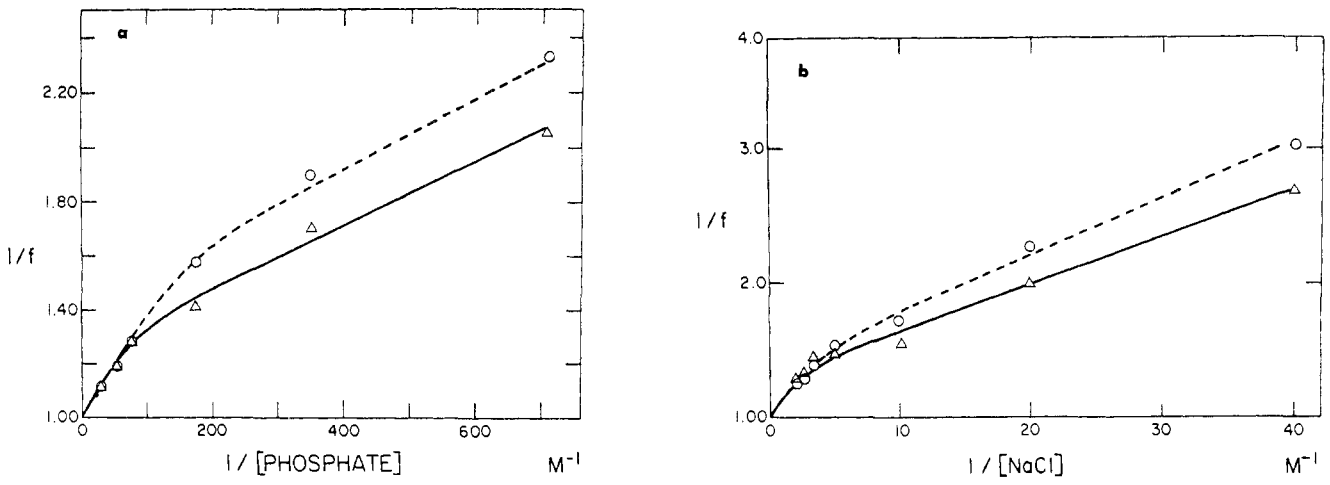

FIGURE 8: (a) Plots of $1/f$ *vs.* the reciprocal of phosphate concentration. Upper curve is for data calculated from fluorescence (○) and lower is that for CD measurements (△). (b) Similar data as in Figure 8a, but sodium chloride was used.

by assuming the existence of a number of equivalent salt binding sites on the protein. If f is the fraction of protein molecules undergoing a change, then $f/(1-f)[P] = K$, where K is the effective association constant between salt of concentration [P] and histone. Plots of $1/f$ *vs.* $1/[P]$ must be linear for the equation to represent the data. This linearity has been observed in studies on histone GRK (Li *et al.*, 1972; Wickett *et al.*, 1972) and on histone KAS (D'Anna and Isenberg, 1972). On the other hand, the linearity is not satisfied by histone LAK data.

Figure 8a and b show the double reciprocal plots for sodium phosphate and sodium chloride. The nonlinearity is evident and shows that the two state assumption is wrong. Within the framework of the histone salt binding model, the nonlinearity suggests either (1) that there is more than a single set of independent binding sites, or (2) the salt binding at one site on the protein affects subsequent salt–histone interaction.

In order to obtain some measure of the relative salt sensitivity of histones LAK, GRK, and KAS, we have tabulated the salt concentrations necessary to induce an f value of 0.50 (Table I).

In a previous section, we noted that the CD changes induced by sodium chloride and sodium phosphate are, essentially, random coil to α-helix formation for some percentage of the total number of residues. If we extrapolate the CD at 220 nm to infinite salt concentration, we may estimate the number of residues involved in the conformational change (Li *et al.*, 1972). This assumes that in the extrapolated state all of the molecules will be in the salt altered form. The extrapolated values and the number of residues undergoing change are given in Table II. Extrapolated values for sodium chloride are consistently 3–5 residues greater than those obtained for sodium phosphate for the same batch of histone. A similar qualitative result has been obtained for the fast change of histone GRK (Wickett *et al.*, 1972), but not for histone KAS where NaCl and sodium phosphate induce nearly the same degree and type of conformational change (D'Anna and Isenberg, 1972).

Chen *et al.* (1972) have obtained standard CD basis spectra by decomposing the CD spectra of proteins of known structure into α-helical, β-sheet, and random coil spectra. For an α helix, they obtain $\Delta\epsilon_{min} = -9.50\ M^{-1}\ cm^{-1}$ which may be compared with $\Delta\epsilon = -10.81\ M^{-1}\ cm^{-1}$ for polylysine (Greenfield and Fasman, 1969). If, instead of polylysine, we were to use the values of Chen *et al.* (1972) to compute a standard difference spectrum, we would raise our estimate of the α-helical content by about 15%.

Discussion

The distinctive feature of histone LAK as compared to KAS and GRK is its marked sensitivity to salt and pH changes.

Even small amounts of salt induce conformational changes (Table I). Furthermore, in contrast to results on histones KAS and GRK, the reciprocal plots of Figure 8 show nonlinearities. Nonlinearity implies that small concentrations of salt induce changes that are qualitatively different from those induced by large concentrations.

TABLE I: Salt Concentration Required to Induce 50% of the Total Salt-Induced Conformational Changes.

Salt	Histone GRK (fast step)	Histone KAS	Histone LAK
NaCl	0.385[a]	0.20[b]	0.060[c]
Phosphate	0.012[a]	0.0045[b]	0.0019[c]

[a] Calculated from the equilibrium constants of Wickett *et al.* (1972). [b] Calculated from the equilibrium constants of D'Anna and Isenberg (1972). [c] From plots of f as a function of salt concentration.

TABLE II: α-Helical Content of the Salt-Altered Conformation of Histone LAK Induced by Phosphate and Chloride, pH 7.0.

Salt	$-[\Delta\epsilon(\infty) - \Delta\epsilon(0)^a]$	% α Helix	Residues α Helix
Phosphate	2.25–0.85[b]	14	18.0
	2.12–0.85[c]	12.7	16.4
NaCl	2.60–0.85[b]	17.5	22.6
	2.35–0.85[c]	15.0	19.4
$MgCl_2$	2.30–0.85[c]	14.5	18.7

[a] $\Delta\epsilon(0)$ was taken as the value of the random coil at 1.0×10^{-4} M HCl. [b] From a single batch of histone ($c = 1.0 \times 10^{-5}$ M). [c] From a single batch of histone different from *b* ($c = 0.5 \times 10^{-5}$ M).

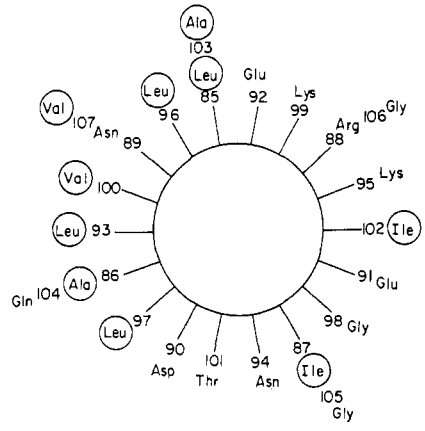

FIGURE 9: Helical wheel of residues 85–107 of histone LAK.

Sensitivity is also observed in pH titrations. Titration from pH 2.0 to 7.0 increases the fluorescence anisotropy sigmoidally. This shape suggests that a conformational change accompanies titration of the acidic groups. This result is different from the behavior of histone KAS (D'Anna and Isenberg, 1972) which shows only relatively small and monotonic changes in that pH region. It is interesting that, while the anistropy curves of KAS and LAK differ appreciably, the intensity curves are nearly identical.

The sensitivity of histone LAK is reflected in quantitative changes seen in results obtained from one preparation to another. These differences are illustrated in Table II where different batches of histones gave different helical contents in the extrapolated salt induced state. It should be noted, however, that all batches showed only one band in gel electrophoresis and their amino acid analyses agreed closely with one another and with the amino acid sequence. The variability could well be due to the marked sensitivity of histone LAK to environmental conditions as shown by the results on salt and pH changes. It could, for example, be due to varying small quantities of an unknown substance, but we have no evidence for this. In any case, because of this variability, the measurements of CD and anisotropy have an uncertainty of the order of 10%. However, despite this variability, for any given histone preparation, we have found that the difference in the measured values, from a reference state of the protein at pH 7.0 in water, has the same shape, as a function of salt or pH, for all histone preparations. In other words, such data differ only by a scaling factor from batch to batch. All of the protein used in this work had the same CD spectra, qualitatively and quantitatively, in water and cacodylate.

We have noted that histone LAK shows a slow increase in anisotropy over a period of a day, while no change in CD was observed in this time. This suggests that there is a slow aggregation of histone LAK which limits the rotational freedom of the tyrosines but does not alter the secondary structure.

Although histones LAK, KAS, and GRK have different sensitivities to salt, the conformational changes of LAK, KAS, and the fast step of GRK all involve a structural change in which roughly the same number of residues go into an α helix. In each case, about 16–23 residues of α helix are formed.

We have previously applied the helix breaking rules of Wu and Kabat (1971) and the helical wheel analysis of Schiffer and Edmundson (1967) to a prediction of the α-helical content of histones GRK and KAS and found a good agreement between the predicted and experimental values. We have done the same thing to histone LAK using an up dated set of statistics (Kabat and Wu, 1973). There is a proline at position 80, a marginal helix breaker at position 105, and a strong helix breaker at 108. Examination of the helical wheel for this region (Figure 9) shows strong potential for α-helix formation from residues 85 to 105, possibly extending to residue 108. An examination of the rest of the protein showed no other region with a strong potential for helix formation. We therefore predict 21–24 residues in an α-helical conformation in good agreement with the observed value.

Acknowledgment

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References

Barclay, A. B., and Eason, R. (1972), *Biochim. Biophys. Acta 269*, 37.

Boublik, M., Bradbury, E. M., and Crane-Robinson, C. (1970a), *Eur. J. Biochem. 14*, 486.

Boublik, M., Bradbury, E. M., Crane-Robinson, C., and Johns, E. W. (1970b), *Eur. J. Biochem. 17*, 151.

Bradbury, E. M., Crane-Robinson, C., Goldman, H., Rattle, H. W. E., and Stephens, R. M. (1967), *J. Mol. Biol. 29*, 507.

Bradbury, E. M., Crane-Robinson, C., Phillips, D. M. P., Johns, E. W., and Murray, K. (1965), *Nature (London) 205*, 1315.

Chen, Y. H., Yang, J. T., and Martinez, H. M. (1972), *Biochemistry 11*, 4120.

D'Anna, Jr., J. A., and Isenberg, I. (1972), *Biochemistry 11*, 4017.

D'Anna, Jr., J. A., and Isenberg, I. (1973), *Biochemistry 12*, 1035.

Diggle, J. H., and Peacocke, A. R. (1971), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 18*, 138.

Edwards, P. A., and Shooter, K. V. (1969), *Biochem. J. 114*, 227.

Greenfield, N., and Fasman, G. D. (1969), *Biochemistry 8*, 4108.

Huberman, J. A. (1973), *Annu. Rev. Biochem. 42*, 355.

Jirgensons, B., and Hnilica, L. S. (1965), *Biochim. Biophys. Acta 109*, 241.

Johnson, W. C., Jr. (1971), *Rev. Sci. Instrum. 42*, 1283.

Kabat, E. A., and Wu, T. (1973), *Biopolymers 12*, 751.

Li, H. J., Wickett, R. R., Craig, A. M., and Isenberg, I. (1972), *Biopolymers 11*, 375.

Panyim, S., and Chalkley, R. (1969), *Biochemistry 8*, 3972.

Schiffer, M., and Edmundson, H. B. (1967), *Biophys. J. 7*, 121.

Shih, T. Y., and Fasman, G. D. (1971), *Biochemistry 10*, 1675.

Small, E. W., Craig, A. M., and Isenberg, I. (1973), *Biopolymers 12*, 1149.

Smerdon, M. J., and Isenberg, I. (1973), *Biochem. Biophys. Res. Commun. 55*, 1029.

Sugano, N., Olson, M. O. J., Yeoman, L. C., Johnson, B. R., Taylor, G. W., Starbuck, W. C., and Busch, H. (1972), *J. Biol. Chem. 247*, 3589.

Taun, D. Y. H., and Bonner, J. (1969), *J. Mol. Biol. 45*, 59.

Tiffany, M. L., and Krimm, S. (1969), *Biopolymers 8*, 347.
Wickett, R. R., and Isenberg, I. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 2687.
Wickett, R. R., Li, H. J., and Isenberg, I. (1972), *Biochemistry 11*, 2952.
Wu, T. T., and Kabat, E. A. (1971), *Proc. Nat. Acad. Sci. U. S. 68*, 1501.
Yeoman, L. C., Olson, M. O. J., Sugano, N., Jordon, J. J., Taylor, C. W., Starbuck, W. C., and Busch, H. (1972), *J. Biol. Chem. 247*, 6018.

Interactions of Histone LAK (f2a2) with Histones KAS (f2b) and GRK (f2a1)†

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ABSTRACT: Measurements of fluorescence anisotropy, relative fluorescence intensity, and circular dichroism (CD) indicate that histones LAK (IIb1; f2a2) and KAS (IIb2; f2b) form a 1:1 complex in solutions of sodium phosphate or sodium chloride, pH 7.0. The order of addition of histone LAK, histone KAS, or salt is not important. The complex is strong and has an association constant of about 10^6 M^{-1}. Upon complexing the number of α-helical residues increases by about 15. The addition of urea reduces complexing. The properties of the LAK–KAS complex are compared with those of KAS–GRK (IIb2–IV) [D'Anna, J. A., Jr., and Isenberg, I. (1973), *Biochemistry 12*, 1035]. Histones LAK and GRK also complex, but the interaction is weaker and comparable to histone self-aggregation. Circular dichroic continuous variation curves indicate an equimolar complex. The interaction of histones LAK and GRK interferes with the slow step of histone GRK.

The treatment of chromatin with varying concentrations of urea (Bartley and Chalkley, 1973; Ilyin *et al.*, 1971) appears to disrupt the compactness of the chromatin and change the circular dichroism (CD) in the 270-nm region to nearly that of free DNA. These results suggest that protein–protein interactions are important to chromatin structure. They are in general agreement with the histone self-aggregation models of Bradbury and Rattle (1972) and Hayashi and Iwai (1971). In those models, parts of histones are bound to DNA and other portions are available for histone–histone interactions.

It has been known for several years that histones aggregate upon addition of salts or at extremes of pH (Cruft *et al.*, 1958; Edwards and Shooter, 1969; Boublik *et al.*, 1970; Barclay and Eason, 1972; Diggle and Peacocke, 1971; Li *et al.*, 1972). Also, some workers have alluded to possible interactions between histones of differing primary structure (Cruft *et al.*, 1958; Laurence, 1966; Shih and Bonner, 1970; Edwards and Shooter, 1970). However, only within the last year has the existence of specific cross-complexes been verified and their characterizations begun (Skandrani *et al.*, 1972; D'Anna and Isenberg, 1973; Kelley 1973).

Skandrani *et al.* (1972) reported that histones LAK[1] and KAS interact during guanidine hydrochloride gradient chromatography on Amberlite resin. From column work and amino acid analyses of the LAK–KAS band, Skandrani *et al.* concluded that the histones form an equimolar complex. Kelley (1973) also reported complex formation based on studies of chromatographic fractions of mixed histones.

We recently reported complex formation between histones KAS and GRK (D'Anna and Isenberg, 1973). CD and fluorescence continuous variation curves imply an equimolar complex. On the basis of a dimer complex, the interaction is strong ($K_A = 10^6$ M^{-1}), and there is an increase of 8 residues of α helix in the complex as compared to the individual histones.

This report describes a number of CD and fluorescence properties of the LAK–KAS complex. We present continuous variation curves and calculate association constants. The effects of urea are examined, and estimates are made of the nature and extent of conformational changes upon complexing. We also examine CD and fluorescence properties of the interaction between histones LAK and GRK. These histones also complex with one another, but much more weakly than LAK–KAS or KAS–GRK.

Experimental Section

Materials and Methods. Calf thymus histone LAK was prepared by the method of Sugano *et al.* (1972) as previously described (D'Anna and Isenberg, 1974). Histone GRK was prepared by exclusion chromatography (Mauritzen *et al.*, 1967), and histone KAS was prepared by the method of Senshu and Iwai (1970). The samples used for fluorescence and CD measurements showed no trace of impurity in electrophoresis (Panyim and Chalkley, 1969), and amino acid analyses agreed with the sequences (Yeoman *et al.*, 1972; Iwai *et al.*, 1970; Ogawa *et al.*, 1969; DeLange *et al.*, 1969).

Fluorescence anisotropy, $r = (E - B)/(E + 2B)$, and CD measurements were obtained as previously described (D'Anna and Isenberg, 1973). CD measurements are reported as $\Delta\epsilon = \epsilon(\text{left}) - \epsilon(\text{right})$ in units of cm^{-1} l./mol of residue or as $\Delta\epsilon' = \epsilon'(\text{left}) - \epsilon'(\text{right})$ in units of cm^{-1} l./mol of histone. The fluorescence anisotropy data as a function of time were

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[1] The nomenclature used in this paper is described in Huberman (1973): GRK = f2a1 = IV; LAK = f2a2 = IIb1; KAS = f2b = IIb2; ARE = f3 = III.