Acknowledgment

We thank Mrs. Roswitha Hopkins for assistance in preparing histone ARE, Mr. Robert Howard for amino acid analyses, and Dr. K. E. Van Holde for use of the Durrum JASCO CD recorder.

References

Brandt, W. F., and Von Holt, C. (1972), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 23,* 357.
D'Anna, J. A., Jr., and Isenberg, I. (1972), *Biochemistry 11,* 4017.
D'Anna, J. A., Jr., and Isenberg, I. (1973), *Biochemistry 12,* 1035.
D'Anna, J. A., Jr., and Isenberg, I. (1974a), *Biochemistry 13,* 2093.
D'Anna, J. A., Jr., and Isenberg, I. (1974b), *Biochemistry 13,* 2098.
D'Anna, J. A., Jr., and Isenberg, I. (1974c), *Biochemistry 13,* 4992.
DeLange, R. J., Fambrough, D. M., Smith, E. L., and Bonner, J. (1969), *J. Biol. Chem. 244,* 5669.
DeLange, R. J., Hooper, J. A., and Smith, E. L. (1972), *Proc. Nat. Acad. Sci., U. S. 69,* 882.
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,* 4109.
Hirs, C. H. W. (1967), *Methods Enzymol. 11,* 199.
Hooper, J. A., Smith, E. L., Sommer, K. R., and Chalkley, R. (1973), *J. Biol. Chem. 248,* 3275.
Huberman, J. A. (1973), *Annu. Rev. Biochem. 42,* 355.
Kelley, R. I. (1973), *Biochem. Biophys. Res. Commun. 43,* 1588.
Laurence, D. J. R. (1966), *Biochem. J. 99,* 419.
Li, H. J., and Isenberg, I. (1972), *Biochim. Biophys. Acta 285,* 467.
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.
Panyim, S., Sommer, K. R., and Chalkley, R. (1971), *Biochemistry 10,* 3911.
Ruiz-Carrillo, A., and Allfrey, V. G. (1973), *Arch. Biochem. Biophys. 154,* 185.
Skandrani, E., Mizon, J., Sautiere, P., and Biserte, G. (1972), *Biochimie 54,* 1267.
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.
Smerdon, M. J., and Isenberg, I. (1974), *Biochemistry 13,* 4046.
Tiffany, M. L., and Krimm, S. (1969), *Biopolymers 8,* 347.
White, F. H., Jr. (1967), *Methods Enzymol. 11,* 481.
Wickett, R. R., Li, H. J., and Isenberg, I. (1972), *Biochemistry 11,* 2952.
Wickett, R. R., and Isenberg, I. (1972), *Proc. Nat. Acad. Sci. U. S. 69,* 2687.

A Histone Cross-Complexing Pattern†

Joseph A. D'Anna, Jr., and Irvin Isenberg*

ABSTRACT: The interactions of histone ARE with histones LAK, KAS, and GRK have been studied by fluorescence anisotropy, circular dichroism (CD), and light scattering. ARE and GRK form a very strong 1:1 complex. We verify the result of R. D. Kornberg and J. O. Thomas [(1974), *Science 184,* 865] that the complex is a tetramer. In 8.5 mM phosphate (pH 7.0) the complex has a binding constant of 0.7×10^{21} M^{-3}. Upon complexing, one or both histones undergo a conformational change, and about nine additional residues per tetramer go into an α-helical conformation. Complexing inhibits the slow aggregation of ARE and GRK. Histones ARE and LAK also interact in a 1:1 molar ratio, but there is no increased α helicity upon complexing, and slow aggregation and β-sheet formation occur. ARE and KAS also interact, but, upon complexing, there is no change in the tyrosine rigidity or in the circular dichroism. A pattern of the interactions between LAK, KAS, ARE, and GRK is presented.

We have reported (D'Anna and Isenberg, 1973, 1974a) that histones LAK and KAS, and histones KAS, and GRK, form strong complexes with a 1:1 molar ratio. Histones LAK and GRK also interact to form a 1:1 complex, but more weakly (D'Anna and Isenberg, 1974a). The complexes require salt for stability and, in fact, they were formed by adding salt to histone mixtures in water. One or both of the partner histones in the strong complexes, KAS-GRK and LAK-KAS, show marked conformational changes upon complexing, and the complex shows considerably more α-helical content than the uncomplexed molecules.

Skandrani *et al.* (1972) and Kelley (1973) have also reported a 1:1 complex of histones LAK and KAS, which was isolated by chromatographic fractionation of mixed his-

† From the Department of Biochemistry and Biophysics, Oregon State University, Corvallis, Oregon. *Received June 3, 1974.* This work was supported by U. S. Public Health Service Grant CA-10872.

[1] The nomenclature used in this paper is described in Huberman (1973): GRK = IV = F2a1, KAS = IIb2 = F2b, LAK = IIb1 = F2a2, ARE = III = F3.

tones.

Very recently Kornberg and Thomas (1974) reported that an ARE-GRK tetramer is obtained by the salt extraction procedure of Van der Westhuyzen and Von Holt (1971). We now report that very strong complexes of ARE and GRK may be formed by individual histones prepared by customary acid extraction procedures. These complexes are tetramers with association constants of 0.7×10^{21} M^{-3}. Upon complexing the α-helical content increases.

Histone LAK also complexes with ARE but without an increase in α-helical content. In both ARE-GRK and ARE-LAK the tyrosine rigidity increases. KAS and ARE also interact but the complex has neither increased α-helical content nor altered tyrosine rigidity. With this paper we are able to present a complete cross-complexing pattern of LAK, KAS, ARE, and GRK.

Experimental Section

Histones ARE, GRK, LAK, and KAS from calf thymus were purified by published methods (Ruiz-Carrillo and Allfrey, 1973; Mauritzen *et al.*, 1967; Sugano *et al.*, 1972; Senshu and Iwai, 1970). They were electrophoretically pure (Panyim and Chalkley, 1969), and the amino acid analyses agreed with the sequences (DeLange *et al.*, 1972, Ogawa *et al.*, 1969; Yeoman *et al.*, 1972; Iwai *et al.*, 1970).

Histone ARE was reduced prior to measurements (D'Anna and Isenberg, 1974c); the other histones were dissolved in water. Concentrations of the histone stock solutions were determined from the absorbance at 275.5 nm (D'Anna and Isenberg, 1972, 1973, 1974b,c). Molar extinction coefficients of 4.04×10^3, 5.4×10^3, 4.05×10^3, and 6.7×10^3 cm^{-1} M^{-1} were used for histones ARE, GRK, LAK, and KAS, respectively.

Solutions for continuous variation measurements were prepared from separate histone stock solutions. Series of solutions were prepared, by pipeting, such that the sum of the concentrations of the two histones after dilution equaled a constant value, C_0. All solutions were diluted with phosphate buffer, and the final pH was 7.0 unless otherwise stated. Because histone ARE or histone GRK alone undergoes slow conformational changes (Li *et al.*, 1972; D'Anna and Isenberg 1974c), the points on the continuous variation curves were obtained by extrapolating the observables to zero time after adding phosphate buffer (D'Anna and Isenberg, 1973). As will be seen, at 1:1 stoichiometry ARE and GRK show no slow change in the complex; therefore, extrapolation is not needed here.

Fluorescence anisotropy and CD were measured as described by D'Anna and Isenberg (1973), and light scattering at 365 nm was measured as described by Smerdon and Isenberg (1973). CD measurements are reported as $\Delta\epsilon$ in units of cm^{-1} l./mol of residue or as $\Delta\epsilon'$ in units of cm^{-1} l./mol of protein.

The equations relating fluorescence and CD continuous variation data to stoichiometry of the complexes have been given (D'Anna and Isenberg, 1973, 1974a). Let F be the fluorescence intensity, and r the anisotropy. Let $\theta = (1 - 10^{-A})/2.3A$ where A is the absorbance of the sample at the excitation wavelength. Then for a complex $A_{n1}B_{n2}$, the variables $\Delta\epsilon' - \Delta\epsilon_I'$, $(Fr - F_I r_I)/\theta$, and $(F - F_I)/\theta$ are directly proportional to the concentration of the complex. The subscript, I, denotes values of the variables expected for noninteracting mixtures. We note that nonzero values of $r - r_I$ indicate that A and B interact, but this can serve only as a crude indicator of stoichiometry. $r - r_I$ is not directly proportional to the concentration of the complex unless the fluorescence intensity is constant at all values of the molar ratio of histone. It follows that, in general, $r - r_I$ cannot be used to obtain association constants.

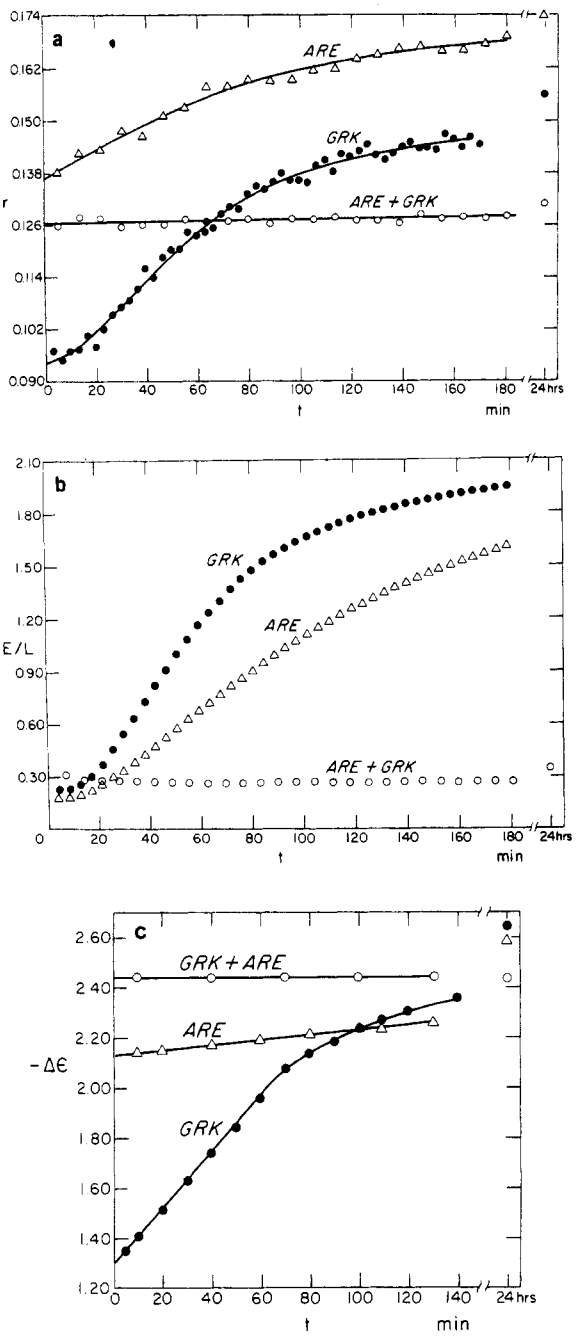

FIGURE 1: (a) Fluorescence anisotropy, (b) light scattering, and (c) CD of histone ARE (Δ), histone GRK (●), and histones ARE plus GRK (○) as functions of time after the addition of phosphate buffer. The final concentration of buffer is 0.016 M. Histone concentrations are 1.0×10^{-5} M in the individual histone solutions and 1.0×10^{-5} M each in the mixed solution.

Results

The ARE-GRK Complex. Histones ARE and GRK form a very strong 1:1 complex in the presence of phosphate as Figure 1 clearly shows. We see that, although both GRK and ARE alone exhibit a slow aggregation, a 1:1 mixture does not. In the complex, GRK and ARE quench each other's slow step. This quenching is similar to that found in the KAS-GRK complex where KAS also quenches the slow

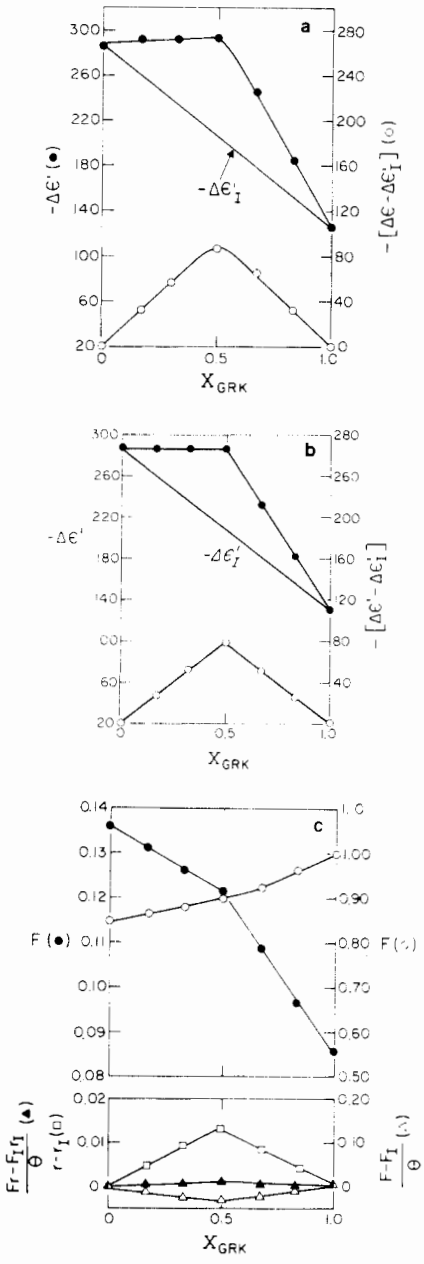

FIGURE 2: Fluorescence and CD continuous variation curves of histones ARE plus GRK at 0.0085 M phosphate, pH 7.0. (a) CD, C_0 = 0.5×10^{-5} M; (b) CD, C_0 = 1.0×10^{-5} M; and (c) fluorescence, C_0 = 1.0×10^{-5} M. X_{GRK} is the mole fraction of histone GRK.

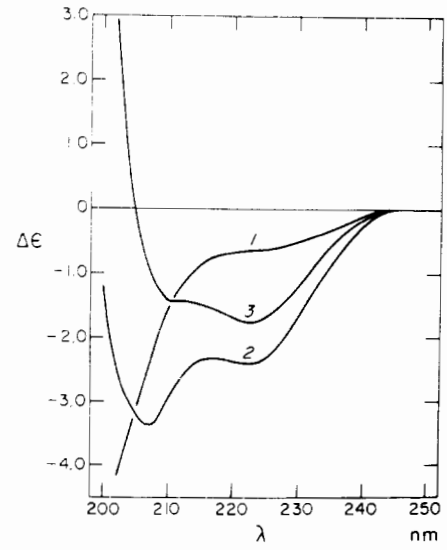

FIGURE 3: CD spectra of ARE and GRK at 0.5×10^{-5} M each in 1.0 $\times 10^{-3}$ M HCl (1) and in 0.020 M phosphate (2). The difference spectrum (3) is computed by subtracting 1 from 2.

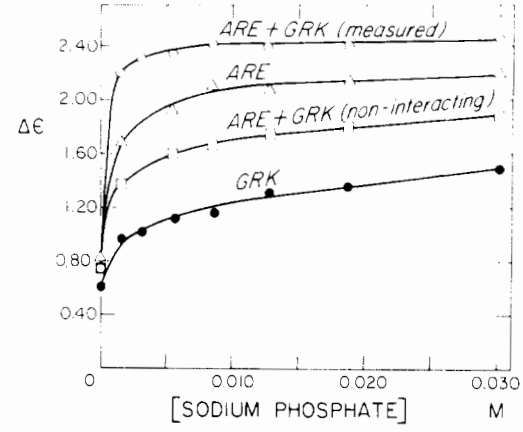

FIGURE 4: CD (220 nm) of the fast step of histone ARE (Δ), $C_0 = 0.5 \times 10^{-5}$ M; the fast step of histone GRK (Δ), $C_0 = 0.5 \times 10^{-5}$ M; and the CD of ARE plus GRK (○), $C_0 = 0.25 \times 10^{-5}$ M each, as functions of phosphate concentration.

step of GRK (D'Anna and Isenberg, 1973).

Instantaneous increases occur in CD and fluorescence properties upon complexing, and these are shown in the continuous variations curves of Figure 2. The continuous variations curves show that histones ARE and GRK complex strongly in a molar ratio of 1:1.

We have made high speed equilibrium sedimentation runs on 1:1 mixtures and obtain a molecular weight of 50,500. Although this value is uncorrected for Donnan effects, it clearly indicates the formation of tetramers $(ARE)_2(GRK)_2$, verifying the report of Kornberg and Thomas (1974).

We have used the method of Schaeppi and Treadwell (1948) to calculate association constants but the binding is so strong that at $C_0 = 1.0 \times 10^{-5}$ M (Figures 2b and c), the curves are triangular, and we are unable to calculate association constants. At $C_0 = 5.0 \times 10^{-6}$ M, complexing is still nearly complete, but we are now able to estimate $K = 0.7 \times 10^{21}$ M^{-3} for an equilibrium between monomers and tetramers.

Secondary Structure of the ARE-GRK Complex. CD spectra and CD difference spectra (Li *et al.*, 1972) of 1:1 mixtures of ARE and GRK are given in Figure 3. The spectrum in acid solution, curve 1, resembles that of a random coil as do the spectra of the individual histones (Li *et al.*, 1972; D'Anna and Isenberg, 1974c). In phosphate buffer at neutral pH, the complex forms. Comparison of the difference spectrum with standard curves (D'Anna and Isenberg, 1972) indicates that some α helix is induced upon complexing, and the α-helical content is larger than that of the histones examined individually. We estimate that the complex has about nine residues of α helix per tetramer in excess over that formed in the fast conformational change of the uncomplexed histones (Figure 4, Table I). The values of Table I were obtained by extrapolation of the data in Figure 4 to infinite phosphate, a procedure which has been described previously (D'Anna and Isenberg, 1973).

Previous work on histone complexes has also shown that complexing increases the α-helical content of the histones.

TABLE I: Percentage of α Helix Induced by Phosphate or by Phosphate and Complexing.

	$-[\Delta\epsilon(\infty) - \Delta\epsilon(R)]^a$	% α Helix	Residues α Helix
ARE	2.45 − 0.92	15.3	21 (lf 135)
GRK	2.12 − 0.60	15.2	15 (of 102)
ARE + GRK (noninteracting)	2.30 − 0.78	15.2	72 (of 474)
ARE + GRK (meas)	2.45 − 0.74	17.1	81 (of 474)

[a] The CD is at 220 nm; $\Delta\epsilon(R)$ is the random coil value in 1.0×10^{-3} M HCl.

TABLE II: α-Helical and β-Sheet Content of Mixed Solutions of Histones ARE plus KAS or of ARE plus LAK in 14 mM Phosphate after Adding Phosphate.

Mixed Solution	Type of Conformational Change[a]	Residues of α Helix per Pair of Histone Molecules	Residues of β Sheet per Pair of Histone Molecules
LAK[b] + ARE (noninter)		26 ± 4	33 ± 8
LAK + ARE (meas)	R → 50% α + 50% β	26 ± 3	26 ± 8
KAS[d] + ARE[c] (noninter)		22 ± 4	33 ± 8
KAS + ARE (meas)	R → 38% α + 62% β	25 ± 2	41 ± 11

[a] R for random coil, α for α helix, and β for β sheet; this classification is based on the difference spectrum technique (Li *et al.*, 1972; D'Anna and Isenberg, 1972). [b] The α-helical content of LAK is taken from D'Anna and Isenberg, 1974b. [c] The α-helix and β-sheet contents of ARE are taken from D'Anna and Isenberg, 1974c. [d] The α-helix values of KAS are taken from D'Anna and Isenberg, 1972.

LAK-KAS has 15 per pair of histones, and KAS-GRK has 8 additional α-helical residues per pair of histones (D'Anna and Isenberg, 1973, 1974a).

The ARE-LAK Complex. The continuous variation plots of Figure 5a show that ARE and LAK form a 1:1 complex. We do not know the number of histone molecules in the complex; but if we assume that a dimer forms, we may estimate a binding constant of the order of 10^5–10^6 M^{-1}. We note that there is a relatively large experimental error here because the absolute values of the changes in the fluorescence properties are small. Furthermore, a CD continuous variation plot at 220 nm shows that there is little or no change in α-helical content upon complexing, and data at other wavelengths confirm this.

Histone LAK inhibits, but does not stop, the slow aggregation of ARE (Figure 6a). At the end of 24 hr considerable β sheet has formed, as in ARE alone (Figure 7). The amount of α helix and β sheet after 24 hr is not significantly different from that measured in individual histone solutions (Table II). We do not know if the aggregation of ARE disrupts the complex or if the complex itself takes part in the aggregation.

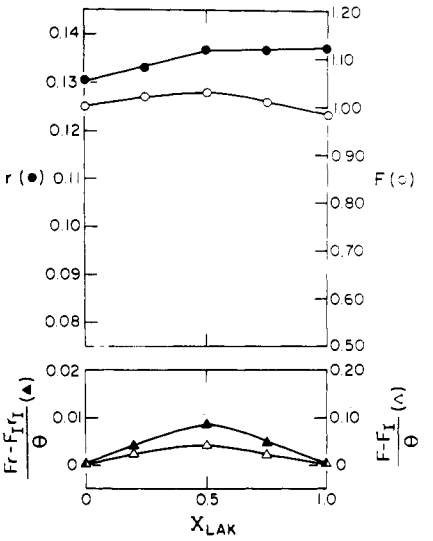

FIGURE 5: Fluorescence continuous variation measurements of ARE plus LAK, $C_0 = 1.0 \times 10^{-5}$ M. X_{LAK} is the mole fraction of histone LAK.

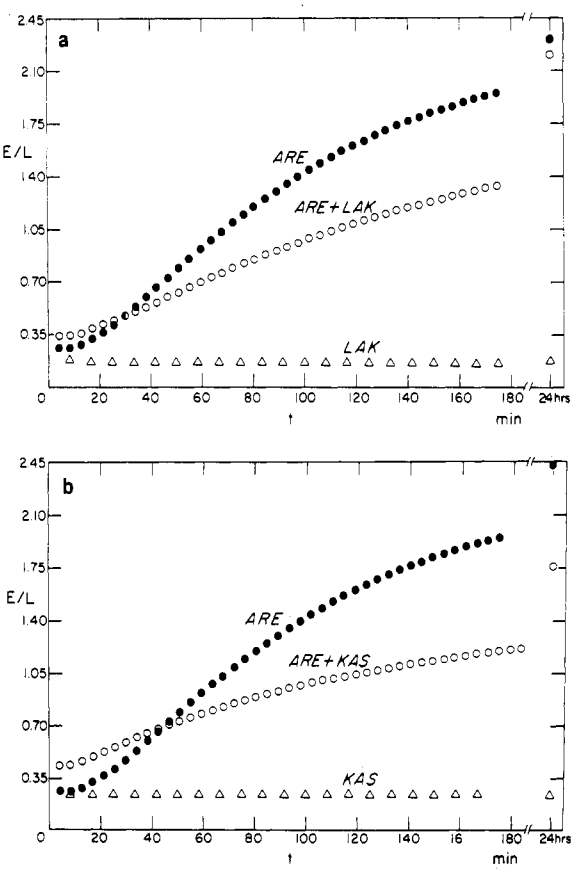

FIGURE 6: Light scattering as a function of time: (a) histone ARE (●), 1.0×10^{-5} M, histone LAK (Δ), 1.0×10^{-5} M, and histones ARE plus LAK (○), 1.0×10^{-5} M each; (b) histone ARE (●), 1.0×10^{-5} M, histone KAS (Δ), 1.0×10^{-5} M, and histones ARE plus KAS (○), 1.0×10^{-5} M each.

ARE-KAS Interaction. Histone KAS inhibits the aggregation of ARE (Figure 6b), but there is little or no change in the anisotropy or CD upon complexing. In addition, we note that the CD spectra after 24 hr (Figure 8) are about the same as that measured for individual histone solutions

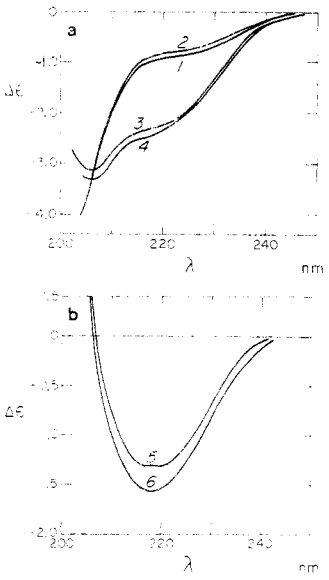

FIGURE 7: (a) CD spectra of histones ARE plus LAK in 1.0×10^{-3} M HCl (1) and in 0.015 M phosphate, pH 7.0 (3); and of ARE plus KAS in 1.0×10^{-3} M HCl (2) and in 0.015 M phosphate (4). The concentration of each histone is 1.0×10^{-5} M, and the spectra were measured with a 1.0-mm cell. (b) CD difference spectra computed from those in Figure 8a: ARE plus LAK, 3 − 1 (5), and ARE plus KAS, 4 − 2 (6).

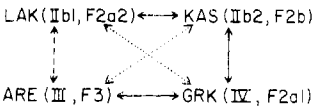

FIGURE 8: Cross-complexing pattern of histones ARE, GRK, LAK, and KAS. The histones which interact strongly are connected by <——> and by (<———>), the others interact more weakly. See text for details.

(Table II). Therefore, while there is interaction, neither the tyrosine rigidity nor the secondary structure changes upon complexing.

Discussion

We are now in a position to summarize the data on all of the complexes of histones LAK, KAS, GRK, and ARE. Figure 8 presents a convenient pattern to help the discussion and Table III summarizes a number of properties.

ARE–GRK, KAS–GRK, and LAK–KAS are all strong complexes. LAK–ARE is also strong, but there is much more experimental uncertainty in determining its strength and it may be somewhat weaker. The first three complexes in Table III have a number of features in common. Complexing inhibits aggregation and β-sheet formation, and there is a considerable increase in the α-helical content upon complexing.

It is interesting that there is a marked change in secondary structure when any of the three strongest complexes form. This implies that there is an induced fit of some sort so that the partners bind to form a compact structure.

Bartley and Chalkley (1972) reported that in the elution of histones from chromatin by increasing concentrations of salt, LAK and KAS dissociated coincidentally, as did ARE and GRK. This suggests that the histones exist as histone cross complexes in the chromatin itself. For if they did not, we would have to imagine that both LAK and KAS dissociated individually in the same range of salt concentration, as did ARE and GRK. In view of the fact that LAK and KAS form a strong complex, as do ARE and GRK, it seems much more reasonable to suppose that the complexes exist in chromatin as such, and that each complex dissociates as a complex from the chromatin at a particular concentration of salt.

TABLE III: Summary of the Cross-Reactions between ARE, GRK, KAS, and LAK.

Complex	α-Helix Formation upon Complexing	Molar Ratio	K	Time-Dependent β-Sheet Formation?
ARE–GRK[d]	9	1:1	0.7×10^{21} M^{-3}	No
LAK–KAS[a]	15	1:1	10^6 M^{-1}	No
KAS–GRK[b]	8	1:1	10^6 M^{-1}	No
ARE–LAK	0	1:1	$0.1–1 \times 10^6$ M^{-1}	Yes
LAK–GRK[a]	1	1:1	0.04×10^6 M^{-1}	Partially[c]
ARE–KAS	0	?	?	Yes

[a] D'Anna and Isenberg, 1974a. [b] D'Anna and Isenberg, 1973. [c] As shown (D'Anna and Isenberg, 1974b) the uncomplexed molecules, but not the complexed ones, aggregate and form β sheet. [d] The association constant for ARE–GRK is that for monomer–tetramer equilibrium and that for LAK–KAS and KAS–GRK for monomer–dimer equilibrium. Monomer–dimer equilibrium is also assumed for ARE–LAK, and LAK–GRK for the purpose of calculating K.

The pattern shown in Figure 8 suggests the speculation that the four histones ARE, GRK, KAS, and LAK might act together as a functional unit in chromatin, and this suggestion is in accord with recent reports in the literature.

Rill and Van Holde (1973) isolated compact nuclease resistant chromatin fragments and Sahasrabuddhe and Van Holde (1974) found them to have a molecular weight of 176,000 with a DNA piece of molecular weight 72,000. The difference is 104,000 while two units each of ARE, GRK, KAS, and LAK have a molecular weight of 109,000. Furthermore, DNA of molecular weight 72,000 has about 116 base pairs and ARE + GRK + KAS + LAK has 110 arginines plus lysines and 11 histidines.

Olins and Olins (1974) reported the observation of spheroid chromatin units in electron micrographs. These units, which they name "ν bodies," are about 70 Å in diameter and may be the same as the nuclease resistent fragments of Sahasrabuddhe and Van Holde, which also have a diameter of about 70 Å (K. E. Van Holde, personal communication).

Kornberg and Thomas (1974) and Kornberg (1974) have also given arguments that the histones exist as complexes in chromatin, based mainly on exchange data (Ilyin *et al.*, 1971) and reconstitution experiments. Kornberg presents a model of chromatin in which there is a repeating unit of 200 base pairs containing two each of histones LAK, KAS, ARE, and GRK.

Of course, at the present time there is as yet no compelling evidence that the complexes exist in chromatin precisely as they do in histone solutions. ARE–GRK, for example, could exist as a tetramer in solution and as a pair of dimers in chromatin. In fact, there is no reason right now to rule out the possibilities that any of the complexes indicated in Figure 8 exist in chromatin. This is, of course, a matter for future work to decide. However, intriguing possibilities are

raised by regarding one LAK-KAS and one ARE-GRK dimer as a four histone linear functional unit regardless of the way in which the dimers might bind with each other and with DNA. Because all four histones are different, the histone unit must have a direction, and therefore could be associated with one of the strands of DNA. The other unit might then exist in a reverse direction and be associated with the other DNA strand. The weaker cross-linking interactions, such as those between ARE and LAK or KAS and GRK, could then help hold the two histone functional units together. The histones would, of course, increase the stability of the DNA duplex.

During replication the weaker interactions might split, but the four histone functional unit might remain associated with its own DNA as separation occurs. The newly synthesized histones would then bind as a new histone functional unit to the new DNA. This picutre is not only consistent with the finding that histones do not turn over during the cell cycle (Byvoet, 1966; Hancock, 1969; Balhorn *et al.*, 1972) but, more importantly, is in accord with the recent work of Tsanev and Russev (1974) who reported that histone replication in regenerating rat liver is semiconservative.

Acknowledgment

We thank Mrs. R. B. Hopkins for preparing the histones, Mr. Robert Howard for amino acid analyses, and Dr. K. E. Van Holde for use of the Durrum-Jasco CD recorder.

References

Balhorn, R., Oliver, D., Hohmann, P., Chalkley, R., and Granner, D. (1972), *Biochemistry 11,* 3915.

Bartley, J. A., and Chalkley, R. (1972), *J. Biol. Chem. 247,* 3647.

Byvoet, P. (1966), *J. Mol. Biol. 17,* 311.

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

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

D'Anna, J. A., Jr., and Isenberg, I. (1974a), *Biochemistry 13,* 2098.

D'Anna, J. A., Jr., and Isenberg, I. (1974b), *Biochemistry 13,* 2093.

D'Anna, J. A., Jr., and Isenberg, I. (1974c), *Biochemistry 13,* 4987.

DeLange, R. J., Hooper, J. A., and Smith, E. L. (1972), *Proc. Nat. Acad. Sci.,* U. S. *69,* 882.

Hancock, R. (1969), *J. Mol. Biol. 40,* 457.

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

Ilyin, Y. Y., Varshavsky, A. Y., Mickelsaar, U. N., and Georgiev, G. P. (1971), *Eur. J. Biochem. 22,* 235.

Iwai, K., Ishikawa, K., and Hayashi, H. (1970), *Nature (London) 226,* 1056.

Kelley, R. I. (1973), *Biochem. Biophys. Res. Commun. 54,* 1588.

Kornberg, R. D. (1974), *Science 184,* 868.

Kornberg, R. D., and Thomas, J. O. (1974), *Science 184,* 865.

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

Mauritzen, C. M., Starbuck, W. C., Saroja, I., Taylor, C. W., and Busch, H. (1967), *J. Biol. Chem. 242,* 2240.

Ogawa, Y., Quagliarotti, G., Jordan, J., Taylor, C. W., Starbuck, W. C., and Busch H. (1969), *J. Biol. Chem. 244,* 4387.

Olins, A. L., and Olins, D. E. (1974), *Science 183,* 330.

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

Rill, R., and Van Holde, K. E. (1973), *J. Biol. Chem. 248,* 1080.

Ruiz-Carrillo, A., and Allfrey, V. G. (1973), *Arch. Biochem. Biophys. 154,* 185.

Sahasrabuddhe, C. G., and Van Holde, K. E. (1974), *J. Biol. Chem. 249,* 152.

Schaeppi, Y., and Treadwell, W. D. (1948), *Helv. Chim. Acta 31,* 577.

Senshu, T., and Iwai, K. (1970), *J. Biochem. 67,* 473.

Skandrani, E., Mizon, J., Sautiere, P., and Biserte, G. (1972), *Biochimie 54,* 1267.

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, C. W., Starbuck, W. C., and Busch, H. (1972), *J. Biol. Chem. 247,* 3589.

Tsanev, R., and Russev, G. (1974), *Eur. J. Biochem. 43,* 257.

Van der Westhuyzen, D. R., and Von Holt, C. (1971), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 14,* 333.

Yeoman, L. C., Olson, M. O. J., Sugano, N., Jordan, J. J., Taylor, C. W., Starbuck, W. C., and Busch, H. (1972), *J. Biol. Chem. 247,* 6018.