CHROMATIN and CHROMOSOME STRUCTURE

Edited by

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PREFACE

During the past decade, great progress has been made in our knowledge of the chemistry and interactions of chromosomal components as well as in the physical structure and biological functions of chromatin and chromosomes. Chromatin and chromosomes in eukaryotic cells have been found to control growth, mitosis, differentiation, hormone action, aging, cancer, and many other phenomena in a higher organism. Such control is accomplished through interactions among chromosomal macromolecules, DNA, histones, nonhistone proteins, and RNA, and through interactions between chromatin and nonchromosomal molecules.

For the benefit of both faculty and students in the Biology Ph.D. Program of the City University of New York (CUNY), a seminar course was offered in the spring of 1975. The speakers emphasized the importance of the various subjects to research investigators and students in biology, biochemistry, biophysics, and biomedical sciences in general. This series of seminars was presented at Brooklyn College and was televised throughout the various campuses of CUNY and other institutions in the metropolitan area.

The coordinators and contributors to this seminar course prepared their seminars as chapters for this book. Most of the chapters were prepared in the spring and summer of 1976 and included many new observations reported after the seminars. In order to include most of the subjects originally presented in the series, Dr. A. G. Harford contributed a chapter on polytene chromosome structure, originally given by Dr. C. Laird, and Dr. H. J. Li contributed two chapters on conformational studies of histones and chromatin subunits, originally covered by Drs. E. M. Bradbury and G. Felsenfeld, respectively.

We are grateful to our former colleagues, Professors L. G. Moriber, D. D. Hurst, and M. Gabriel who helped in making possible the seminar series. We thank our former students Drs. C. Chang, I. M. Leffak, M. F. Pinkston, R. M. Santella, S. S. Yu, and Mr. J. C. Hwan and N. Rubin who have provided assistance in the preparation of lecture transcripts. Our thanks to Mrs. R. Bellamy and Mrs. D. Galeno, who spent many hours in typing the manuscripts and lecture transcripts.

NOMENCLATURE FOR HISTONE FRACTIONS

Lysine-rich histones	H1	I	F1
	H5	V	F2c
Slightly lysine-rich histones	H2A	IIb1	F2a2
	H2B	IIb2	F2b
Arginine-rich histones	H3	III	F3
	H4	IV	F2a1

CONTENTS

Chapter 1

CONFORMATIONAL STUDIES OF HISTONES

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I. Introduction

The regular supercoiled structure with a pitch of 120Å and a diameter of 100Å, as originally proposed by Pardon *et al.* (1), is not compatible with either the observations of a string of beads in chromatin (2-5) or the separation of chromatin into nuclease-susceptible and nuclease-resistant fragments (6-19). A new concept of chromatin structure, namely a chromatin with distinct subunits, has been developed. Recently, quite a few models have been proposed to describe these subunits (20-24). The subject of chromatin structure has been dealt with extensively in a recent review (25).

In the past six years, studies of histone conformation and histone-histone interactions in solution have greatly increased the understanding of the structures and interactions among histones; this information is a necessary prerequisite for research on detailed structures of histones and on histone assembly into chromatin subunits. Such studies include kinetic and equilibrium interactions and conformations using circular dichroism (CD), fluorescence and nuclear magnetic resonance (NMR).

II. Circular Dichroism (CD) and Fluorescence Studies of Histone H4

Initially, formation of α-helix in histones upon the addition of salt or DNA were investigated by optical rotatory dispersion (ORD) (26-28). Jirgenson and Hnilica (26) reported phosphate to be more efficient than chloride in inducing an ordered structure in histones. Subsequently, more extensive studies on the conformation and interactions of histone H4, using CD and fluorescence polarization, were combined

with kinetics and equilibrium methods, as described below (29).

The fluorescence anisotropy of histone H4 tyrosine residues in water, r_w, and at time t after the addition of phosphate, $r_p(t)$, is depicted in Fig. 1, which shows a dependence of this anisotropy on both time and phosphate concentration.

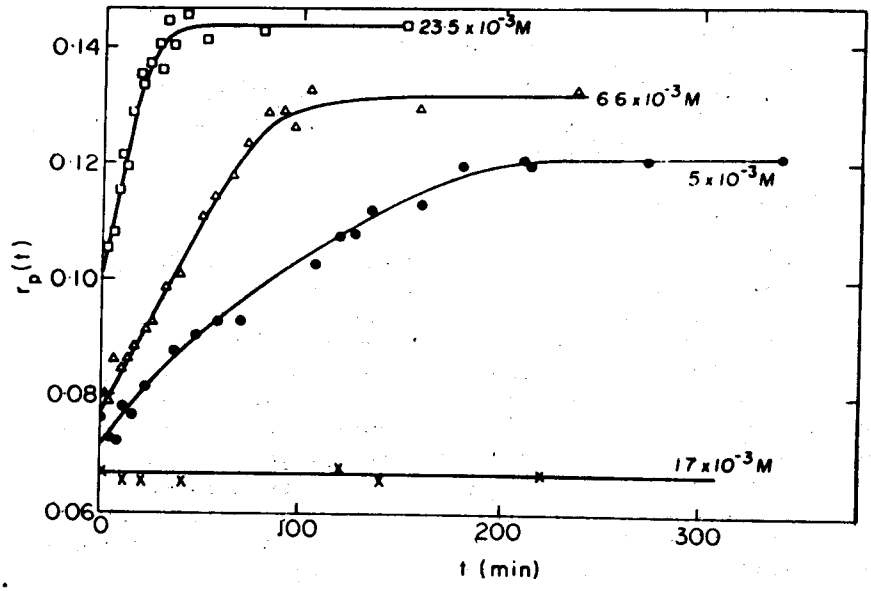

Figure 1. Fluorescence anisotropy of histone H4 as a function of time and phosphate concentrations. H_o *= 0.8 x* 10^{-5} *moles per liter, pH 7.4. Phosphate concentrations are shown in the figure. The anisotropy of histone H4 in water,* r_w *is 0.050 ± 0.003. Li, H. J., Wickett, R., Craig, A. H., and Isenberg, I. (1972)* Biopolymers 11, *375. Reprinted with the permission of John Wiley and Sons, Inc.*

At low phosphate concentration, 1.7 x 10^{-3}M, for example, after a rapid increase from r_w = 0.050 to r_p = 0.067, the fluorescence anisotropy remains unchanged for more than 8 hours. At higher phosphate concentrations, in addition to the rapid increase in r, there is a slow and time-dependent increase which finally reaches a plateau, $r_p(\infty)$. The slow step can be described, approximately, as a single exponential function of time.

$$\frac{r_p(\infty) - r_p(t)}{r_p(\infty) - r_p(0)} = e^{-t/\tau_F} \qquad (1)$$

where τ_F is the time constant for this step of the reaction as measured by fluorescence. The plot of eq. (1), shown in Fig. 2a, yields both the time constant τ_F and $r_p(0)$, the extrapolated anisotropy at t = 0 after the addition of phosphate. With these factors determined, it is then possible to calculate $r_p(0) - r_w$ of the fast step and $r_p(\infty) - r_p(0)$ of the slow step.

As with the anisotropy, the CD was also observed to be a function of time after the addition of phosphate. The CD results can be described by an equation similar to eq. (1). Define $\Delta\varepsilon_w(\lambda)$ and $\Delta\varepsilon_p(t,\lambda)$, respectively, as the CD of amide groups of histone H4 in water and at time t after the addition of phosphate, both measured at wavelength λ. The slow step of the conformational change measured by CD can be described by the following equation:

$$\frac{\Delta\varepsilon_p(\infty,220) - \Delta\varepsilon_p(t,220)}{\Delta\varepsilon_p(\infty,220) - \Delta\varepsilon_p(0,220)} = e^{-t/\tau_{CD}} \qquad (2)$$

where τ_{CD} is the time constant of the slow step measured by CD (29). Fig. 2b shows the results from a plot of eq. (2).

Thus, conformational changes in histone H4 can be separated into a fast and a slow step by measuring either the fluorescence of tyrosine residues or the CD of amide groups. Based upon difference CD spectra, it was further demonstrated that, following the addition of phosphate, the fast step involves α-helix formation and the slow step formation of β-sheet in histone H4 (Fig. 3) (29).

The conformational changes in both fast and slow steps depend not only upon phosphate concentration (Fig. 4) but also upon histone concentration (Fig. 5).

The fast-step conformational changes in Fig. 4 can be shown to result from binding of phosphate to histone H4 by use of the following equations:

$$B + X \underset{\leftarrow}{\overset{K}{\rightarrow}} BX \qquad (3)$$

$$K = \frac{[BX]}{[B]\ [X]} \qquad (4)$$

where [B] is the concentration of binding sites, [X] the concentration of ligand (phosphate in Fig. 4), and [BX] the concentration of bound sites. Table I summarizes the binding

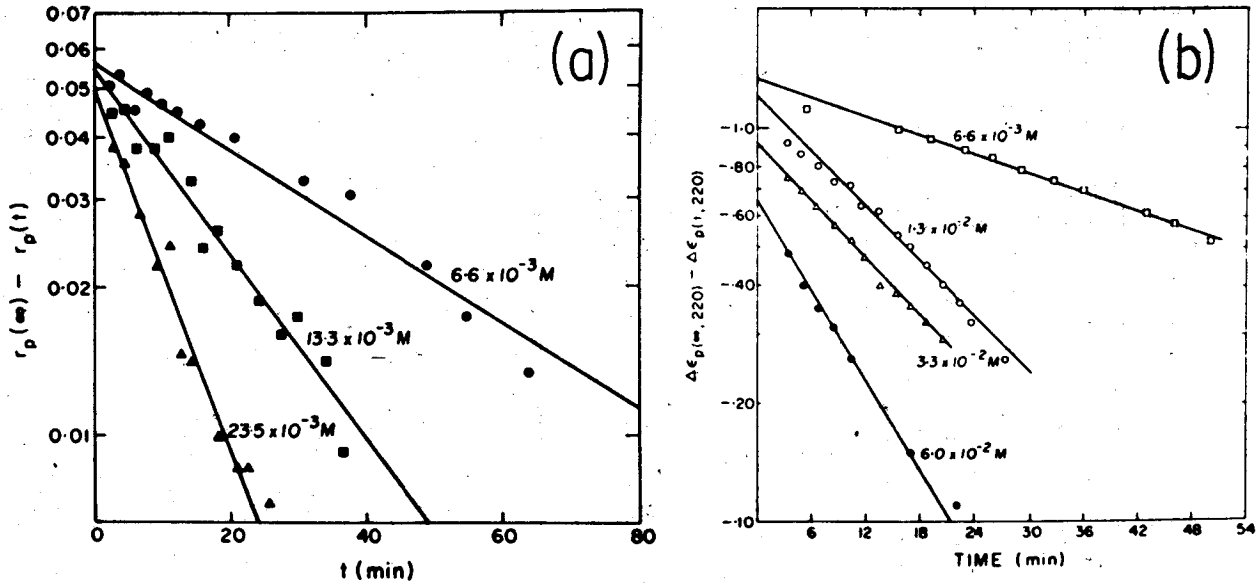

Figure 2a. Semilogarithmic plot of $r_p(\infty) - r_p(t)$ for the slow step as a function of time.

2b. Semilogarithmic plot of $\Delta\epsilon_p(\infty,220) - \Delta\epsilon_p(t,220)$ as a function of time. H_o = 0.8 x 10^{-5}M, pH 7.4; phosphate concentrations are shown. CD results are given as $\Delta\epsilon = \epsilon$(left) - ϵ(right) in cm^{-1} liter per mole of residue. Li, H. J., Wickett, R., Craig, A. M., and Isenberg, I. (1972) Biopolymers 11, *375. Reprinted with the permission of John Wiley and Sons, Inc.*

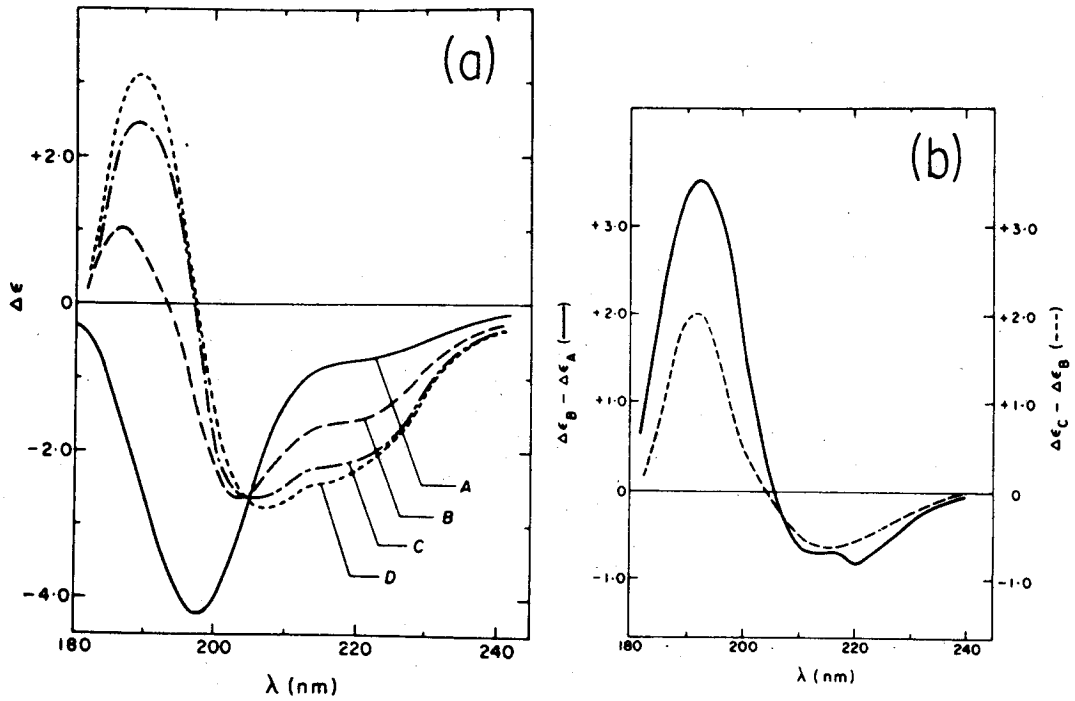

Figure 3a. *CD spectra of histone H4 in water (Curve A), in 3.3 x 10^{-3}M phosphate, pH 7.4, recorded from t = 25 to 55 min (Curve B), from t = 290 to 320 min (Curve C), and from t = 960 to 990 min (Curve D). H_O = 0.8 x 10^{-5}M, optical path length was 1 mm.*

3b. *Difference CD spectra computed from Figure 3a. Li, H. J., Wickett, R., Craig, A. M., and Isenberg, I. (1972)* Biopolymers 11, 375. *Reprinted with the permission of John Wiley and Sons, Inc.*

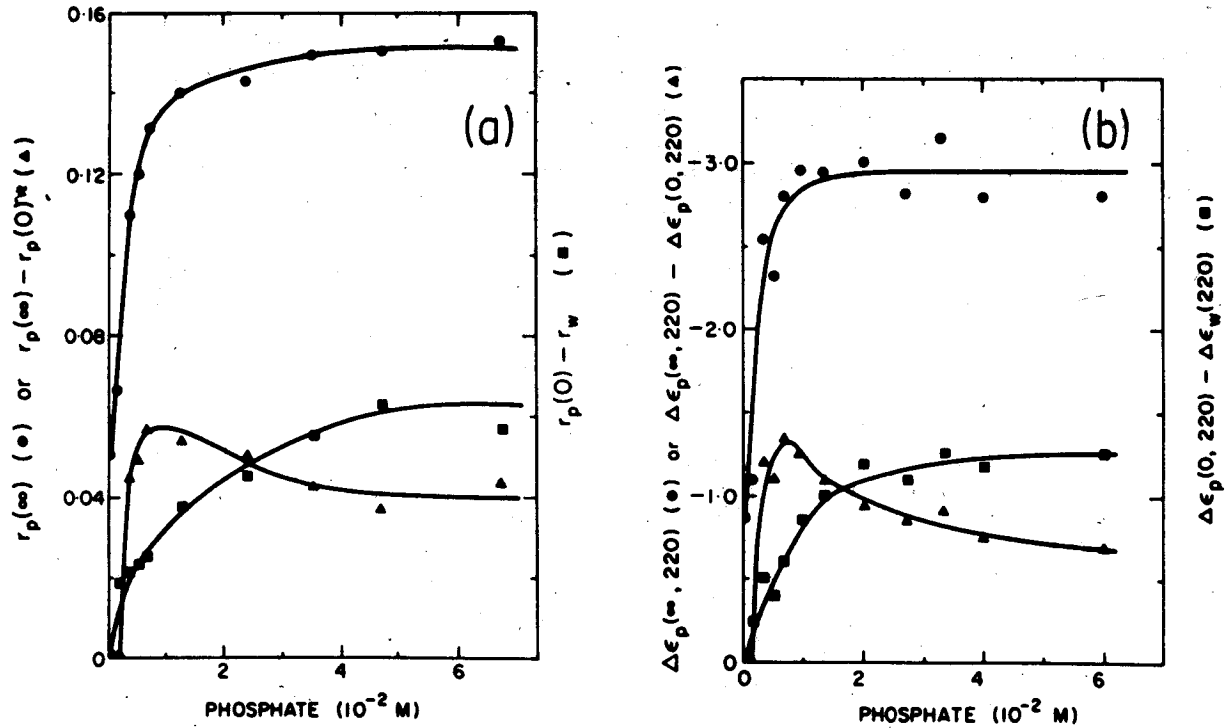

Figure 4a. Phosphate concentration dependence of the final anisotropy and anisotropy changes of the fast and slow processes. $r_w = 0.05 \pm 0.003$.

4b. Phosphate concentration dependence of the final CD and CD changes of the fast and the slow steps. $H_o = 0.8 \times 10^{-5}$M, pH 7.4, measured at 220 nm. $\Delta\epsilon_w(220) = 0.85 \pm 0.1$. Li, H. J., Wickett, R., Craig, A. M., and Isenberg, I. (1972) Biopolymers *11, 375. Reprinted with the permission of John Wiley and Sons, Inc.*

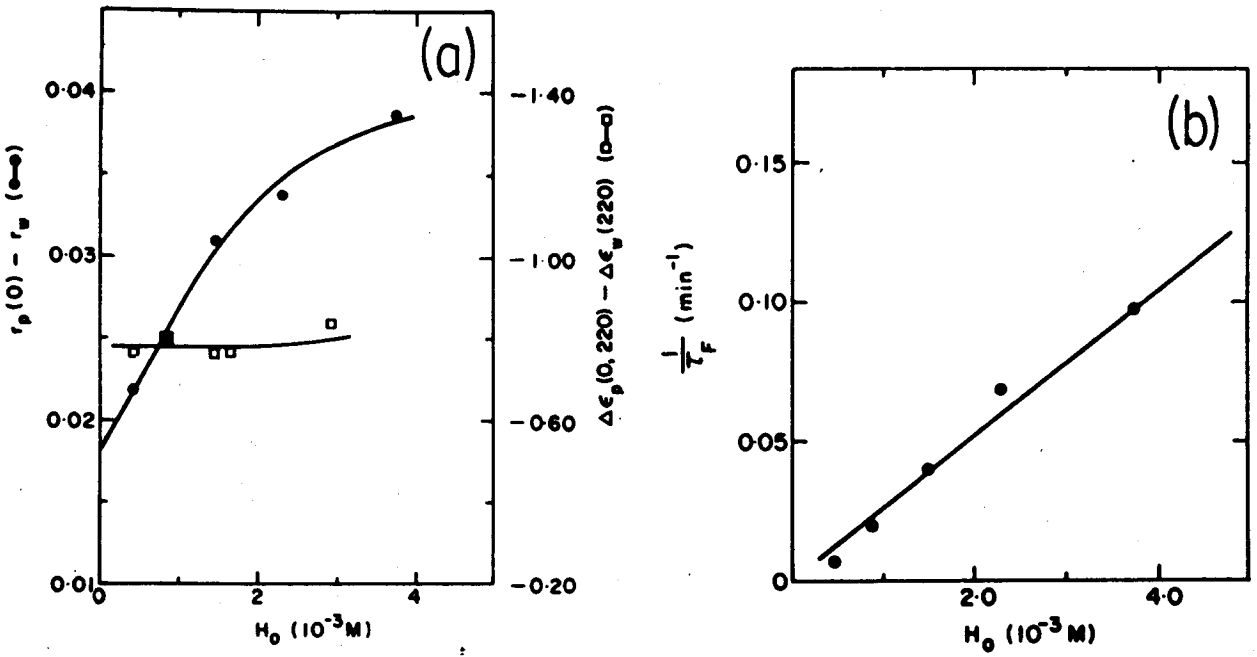

Figure 5. Histone concentration dependence of the anisotropy change and the CD change of the fast step (a) and the rate constant of slow step (b). Phosphate concentration is 6.6 x 10^{-3}M, pH 7.4. The unit of histone concentration in this figure should be divided by 102, the total number of residues in histone H4. Li, H. J., Wickett, R., Craig, A. M., and Isenberg, I. (1972) Biopolymers 11, 375. *Reprinted with the permission of John Wiley and Sons, Inc.*

constants for histones with various salts as ligands. In general, the binding affinity for histone is greater with

Table I. Binding constants between histones and various salts.

Histones	Salt	$K^*_{CD}(M^{-1})$	$K^*_F(M^{-1})$	Reference
H4	Sodium phosphate (pH 7.4)	80	85	29
	NaH_2PO_4 (PO_4^-)	10	12	29,30
	Na_2HPO_4 ($PO_4^=$)	120	130	29
	Na_2SO_4	105	95	30
	$NaClO_4$	32	16	30
	NaCl	2.2	2.6	30
	$MgCl_2$	2.5	3.3	30
	NaF	2.0	2.2	30
H2B	Sodium phosphate (pH 7.4)	150	220	31
	NaCl	5.4	4.3	31
H3	Sodium phosphate	660	710	32

*K_D is measured by CD and K_F measured by fluorescence anisotropy.

divalent anions, $SO_4^=$ or PO_4^-,,than with monovalent, PO_4^- or ClO_4^-,,and these monovalent anions bind with greater affinity than do Cl^- and F^-. The physical meaning of these differences will be discussed later, but it is noted here that the greater efficiency of phosphates over chloride in inducing conformational changes in histones confirms the earlier observation based upon optical rotatory dispersion studies (26).

In the case of histone H4, the dependence of anisotropy on histone concentration (Fig. 5a) was attributed to dimer formation, while the dependence of $1/\tau_F$ on histone H4 concentration (Fig. 5b) was explained only as intermolecular inter-

action in the slow step (29).

III. Mechanism of Conformational Changes in Histone H4

Despite an extensive study on histone H4 conformation by Li *et al.* (29) and Wickett *et al.* (30) and on other histones by Isenberg and co-workers (31-37), the experimental results are still fragmentary and not well integrated. For instance, the exact physical meaning of the plot of eqs. (1) and (2) (Fig. 2a and 2b) and of the linear relationship between $1/\tau_F$ and histone concentration (Fig. 5b) cannot be fully understood in terms of molecular interaction. However, the following approach, made in 1973, may still be useful for this discussion and for those interested in research into more detailed mechanisms of conformational changes in histones in the future.

Define the following terms for the equations:

H: a histone H4 molecule.

X: a salt molecule, presumably an anion.

HX_i: a histone molecule bound by i molecules of X at a certain salt concentration.

$(HX_i)_2$: a dimer of HX_i with α-helical structure but no β-sheet.

$(HX_i)_{2,\beta}$: a dimer of HX_i after β-sheet formation.

$k_{12,i}$ and $k_{21,i}$: forward and backward rate constants of the first step of reaction (dimerization).

$k_{23,i}$: forward rate constant of the second step of reaction.

$K_i = \frac{k_{12,i}}{k_{23,i}}$: equilibrium constant of the first step of reaction (dimerization).

It will be shown below that the following equation can describe the observations reported by Li *et al.* (29):

$$2HX_i \underset{k_{21,i}}{\overset{k_{12,i}}{\rightleftarrows}} (HX_i)_2 \xrightarrow{k_{23,i}} (HX_i)_{2,\beta} \qquad (5)$$

Assume that the first step (dimerization) is rapid compared with the second step (β-sheet formation) and that $K_i(HX_i) \ll 1$. The first assumption has been shown to be true (29); the second will be tested below. Based upon these two assumptions, eqs. (6) and (7) can be derived (see Appendix):

$$(HX_i)_{2,\beta} = \frac{\frac{1}{2}k_{app}(H_o)t}{1 + \frac{1}{2}k_{app}t} \quad (6)$$

where

$$k_{app} = 4k_{23,i}\, K_i(H_o) \quad (7)$$

Eq. (8) defines f, the fraction of histone H4 molecules in β-sheet structure, in terms of $(HX_i)_{2,\beta}$ and (H_o), which are, respectively, the concentration of H4 dimer with β-sheet and the total histone concentration in monomer:

$$f = \frac{2(HX_i)_{2,\beta}}{(H_o)} \quad (8)$$

f can be expressed in terms of changes in either CD or fluorescence anisotropy as a result of the slow step (29). As shown in the Appendix, for CD and for fluorescence anisotropy, the following two equations can be obtained:

$$\frac{1}{\Delta\varepsilon_p(\infty,220) - \Delta\varepsilon_p(t,220)} =$$

$$\frac{1}{\Delta\varepsilon_p(\infty,220) - \Delta\varepsilon_p(0,220)}\,[1 + \tfrac{1}{2}(k_{app})_{CD}t] \quad (9)$$

$$\frac{1}{r_p(\infty) - r_p(t)} = \frac{1}{r_p(\infty) - r_p(0)}\,[1 + \tfrac{1}{2}(k_{app})_F t] \quad (10)$$

As t is small, both eqs. (9) and (10) can be rewritten as:

$$\frac{\Delta\varepsilon_p(\infty,220) - \Delta\varepsilon_p(t,220)}{\Delta\varepsilon_p(\infty,220) - \Delta\varepsilon_p(0,220)} = \exp[-\tfrac{1}{2}(k_{app})_{CD}t] \quad (11)$$

and

$$\frac{r_p(\infty) - r_p(t)}{r_p(\infty) - r_p(0)} = \exp[-\tfrac{1}{2}(k_{app})_F t] \quad (12)$$

Eqs. (11) and (12) would be identical to eqs. (2) and (1), respectively, if

$$\frac{1}{\tau_{CD}} = \tfrac{1}{2}(k_{app})_{CD} \quad (13)$$