The Interaction of Histone IV with Nucleoside Triphosphates

(ATP, GTP, CTP, and TTP/conformational change/fluorescence polarization/
nucleotide–protein interaction/circular dichroism)

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ABSTRACT The nucleoside triphosphates ATP, GTP, TTP, and CTP induce conformational changes in histone IV at concentrations that are of the same magnitude as the histone concentration. The purine nucleoside triphosphates are much more effective than the pyrimidine nucleoside triphosphates.

We have reported (1, 2) that anions induce conformational changes in histone IV. We now report that ATP, GTP, CTP, and TTP also induce conformational changes in histone IV, and furthermore that the nucleotides act at low concentrations, of the same order of magnitude as the histone concentrations themselves.

The interaction of histone IV with ATP or GTP is especially strong since, for these, plots of the intrinsic fluorescence anisotropy as a function of the number of nucleotides per histone are essentially identical over a 4-fold range of histone concentrations. It will also be seen that these plots are sigmoidal, indicating cooperativity.

MATERIALS AND METHODS

Calf-thymus histone IV was prepared by the method of Ogawa et al. (3). Amino-acid analyses agreed with the published sequence (3, 4). Histone concentrations were determined spectrophotometrically with ε280 = 4.7 × 10⁴ cm⁻¹/mmol of residue per liter (5, 6). Hₜ will here denote the total histone concentration in mol of protein per liter.

Nucleoside triphosphates, AMP, and cAMP were purchased from Calbiochem and Sigma Chemical Co. as sodium salts. Concentrated stock solutions were added to histone IV in 5.0 mM cacodylate buffer (pH 6.8) with constant mixing. The sample temperature was maintained at 22.0 ± 0.5°C. The instrument and techniques used for measurement of the intrinsic fluorescence of histone IV have been described (1, 2, 7). Excitation was at 280 nm with vertically polarized light. The anisotropy is defined as \( r = (I_H - I_L)/(I_H + 2I_L) \), where \( I_H \) and \( I_L \) are components of emission parallel to and perpendicular to the excitation beam. The fluorescence intensity was corrected for nucleotide absorbance as described by Parker (8). Circular dichroism (CD) data was obtained on a model CD-SP Durrum-Jasco CD recorder. CD results are reported as \( \Delta \epsilon = \epsilon_l - \epsilon_r \), where \( \epsilon_l \) and \( \epsilon_r \) are extinction coefficients for left- and right-handed circularly polarized light. The units of \( \Delta \epsilon \) are cm⁻¹ liter/mol of residue.

RESULTS

Fluorescence anisotropy changes induced by ATP and GTP

The intrinsic fluorescence emission of histone IV is due to its tyrosines. The anisotropy of this emission is a function of the rate of rotational diffusion of the tyrosine residues and will increase if the tyrosine environment becomes more rigid. We have reported (1, 2) that salt-induced conformational changes in histone IV that led to marked increases in fluorescence anisotropy could be separated into fast and slow components.

Fig. 1 shows the effects of ATP, ADP, and GTP on the anisotropy of the tyrosine emission at \( H_0 = 4.4 \mu M \). A small amount of time-dependent change was observed at concentrations above 15 \( \mu M \) ATP or GTP in the absence of NaCl. In 25 mM NaCl, or with ADP solutions, no time dependence at all was observed over the range observed. The amount of slow change, when it occurs, is small compared to that induced by small anions (1, 2). When small changes were observed, the anisotropy was extrapolated to zero time, as described (1, 2), to obtain the data shown in Fig. 1.

Two features of the curves in Fig. 1 are of note. First, ATP, and GTP are effective in inducing fluorescence anisotropy changes at low concentrations, where the ratio of ATP or GTP to histone is small. Second, the curves have a sigmoidal shape.

![Fig. 1](attachment:image.png)

**FIG. 1.** (a) Fluorescence anisotropy of histone IV in 5.0 mM cacodylate buffer against ATP pH 6.6–6.8. O—O, No NaCl; •—•, 25 mM NaCl; Δ—Δ, ADP, in 5.0 mM cacodylate buffer (pH 6.8). (b) Fluorescence anisotropy of histone IV in 5.0 mM cacodylate buffer (pH 6.8) against GTP. O—O, No NaCl; •—•, 25 mM NaCl.

Abbreviation: CD, circular dichroism.
For a more quantitative picture of the interaction, we proceed as described in work with small ions (2, 7). The fraction of histone molecules that have undergone the conformational change may be defined as (1, 2, 7)

$$f = \frac{I_A(r_A - r_w)}{I_w(r_A - r_w)}$$

where $r_w$ is the anisotropy in water, $I_A$ is the fluorescence intensity ($I_{||} + 2I_{\perp}$), and $r_A$ is anisotropy, at the nucleotide concentration $A$. $I_A$ and $r_A$ are the intensity and anisotropy at infinite nucleotide concentration. To obtain $I_w(r_A - r_w)$, we find it possible to extrapolate a plot of $\ln [I/A(r-w)]$ against $1/A$ to $1/A = 0$. These semilog plots are linear for nucleotide to histone ratios above 1.5, and furthermore extrapolate to the same value for different histone concentrations. The value of $I_w[1/(r_A - r_w)]$ so obtained is 0.111 for ATP and 0.125 for GTP. Separate extrapolations of the relative intensity to infinite nucleotide concentration give $I_w/I_w = 0.77$ for ATP, and 0.70 for GTP, where $I_w$ is the intensity in water. Combination of the two extrapolations gives $r_w - r_w = 0.086$ for ATP and 0.87 for GTP. We found $r_w - r_w = 0.074$ for the fast step induced by sodium phosphate at pH 7.4 (1).

Plots of $f$ against ATP at three histone concentrations are shown in Fig. 2a, while in Fig. 2b and c, we have plotted $f$ as a function of the number of ATP or GTP molecules per histone IV molecule. These latter curves do not differ significantly from one another. Furthermore, the curves for ATP and GTP are close to one another. We note that at nucleotide to histone ratios of about 5.0 the histone begins to precipitate.

### CD difference spectra induced by added ATP and GTP

The circular dichroism spectrum of histone IV is changed markedly by the addition of nucleoside triphosphates (Fig. 3). The change is like the fast change seen upon the addition of small anions (1, 2), but consistent with our anisotropy data, the concentrations of nucleoside triphosphate needed for appreciable change are of the same order of magnitude as the histone concentration.

We find appreciably smaller slow changes than occur upon the addition of small anions (1, 2). At $H_0 = 4.4 \mu M$ we do see small amounts of slow change at nucleotide to histone ratios of 3.0 or higher. However, these slow changes are so much smaller, and faster, than those observed with small anions that we have not yet been able to obtain CD difference spectra for the slow change. At $H_0 = 17.5 \mu M$ no changes were observed after the start of the measurement (1.5 min after ATP additions), even at the highest ATP concentration studied.

The small CD of the nucleotides above 250 nm was unchanged by the addition of histone, and so it seems reasonable to assume that the small CD they possess in the region of protein absorbance is also unchanged by histone. This CD was simply subtracted, therefore, from the histone CD spectrum. The relative contribution of the CD of ATP, at the highest ATP concentration, is shown as the dashed line on Fig. 3.

The CD difference spectra taken at ATP/$H_0 = 2.0$ (1 and 2 of Fig. 3) are very similar in shape to those obtained for the fast conformational change in histone IV (1, 2). They agree quite well with a theoretical difference spectrum for $\alpha$-helix formation (1). At higher ATP concentrations the shape of the difference spectrum changes somewhat. Spectra 3 and 4 have minima at 220 nm but the shoulder at 212 nm is less pronounced. Spectrum 5 ($H_0 = 17.0 \mu M$, ATP = 66 $\mu M$) has a definite shoulder at 216 nm and a crossover point at 208 nm.
is not molecule is triphosphate, but induced The CD change at 220 nm shows plots of \( \Delta \varepsilon \) against ATP/Ho in 5.0 mM cacodylate buffer (pH 6.8). \( H_0 = 17.5 \, \mu M \) (A); \( H_0 = 8.8 \, \mu M \) (B); \( H_0 = 4.4 \, \mu M \) (C); \( H_0 = 4.4 \, \mu M \), pH 5.9 (D). \( \Delta \varepsilon \) shows the value that the CD reaches after the slow change at the lowest histone concentration at ATP/Ho = 4.7. (b) CD change at 220 nm against GTP/Ho. \( H_0 = 17.5 \, \mu M \) (A); \( H_0 = 4.4 \, \mu M \) (C). These perturbations in the \( \alpha \)-helical difference spectrum may be the result of \( \beta \)-sheet formation, as the CD spectrum of the \( \beta \)-sheet has a peak at 216 nm. However, as stated, no time dependence was observed in the CD under these conditions. If \( \beta \)-sheet is formed, it is formed in less than 1.5 min.

Fig. 4 shows plots of \( \Delta \varepsilon (220) \) against nucleotide per histone. In contrast to the fluorescence results, the curves are not superimposable, at least above 1 ATP per histone. Even below 1 ATP per histone, because of experimental error it is difficult to see if the curves are really the same or simply merge as the ATP concentration is lowered. However, as with the fluorescence data, the slope becomes larger at higher ATP concentrations.

Changes induced by CTP and TTP

The CD as a function of added CTP and TTP per histone molecule is shown in Fig. 5a. The change per molecule added is not as great as that found with added purine nucleoside triphosphate, but CTP and TTP have effects that are still much stronger than those of small anions (1, 2). We note that CTP and TTP have equal effects.

These CD data may be interpreted as resulting from equilibrium binding. Plots of \( 1/\Delta \varepsilon - \Delta \varepsilon_{\text{eq}} \) against 1/A above nucleotide per histone ratios of 2.0 are linear, as with small anions. Furthermore, the effective equilibrium constant is the same for CTP and TTP at both \( H_0 = 4.4 \, \mu M \) and \( H_0 = 13 \, \mu M \), namely, \( 1.3 \times 10^4 \text{ M}^{-1} \).

The anisotropy change breaks sharply upward at the higher range of nucleotide concentrations (Fig. 5b). The nucleotide

![Fig. 4. (a) CD change at 220 nm and zero time against ATP/Ho.](image)

![Fig. 5. (a) CD change at 220 nm against CTP/Ho for CTP and TTP. CTP, \( H_0 = 4.4 \, \mu M \) (C); \( H_0 = 13 \, \mu M \) (B); TTP, \( H_0 = 4.4 \, \mu M \) (A); \( H_0 = 13 \, \mu M \) (D); all samples in 5.0 mM cacodylate buffer, final pH 6.6-6.8. (b) Fluorescence anisotropy change against CTP/Ho for CTP and TTP. Symbols as in a.](image)

![Fig. 6. CD difference spectra for CTP and TTP. (1) \( H_0 = 4.3 \, \mu M \), CTP = 17 \, \mu M (---); (2) \( H_0 = 1.3 \, \mu M \), TTP = 52 \, \mu M (--.--); (3) \( H_0 = 13 \, \mu M \), CTP = 52 \, \mu M (-----).](image)
to histone ratio at which this break occurs depends on the histone concentration. A reciprocal-plot analysis of the fluorescence data below the break, ATP/H₀ < 6.0, and at the lower histone concentration, H₀ = 4.4 μM, yielded linear plots and gave K = 1.2 × 10⁴ M⁻¹ for CTP and K = 1.4 × 10⁴ M⁻¹ for TTP.

For the higher histone concentration, H₀ = 13 μM, the sharp break upward occurs at about a ratio of pyrimidine nucleoside triphosphate to histone of only 2.0. The range from 0.0 to 2.0 is too small to be used for accurate reciprocal plots. Consequently, we could not obtain an effective equilibrium constant for these data.

The equilibrium constants obtained from fluorescence data agree with those from CD data.

CD difference spectra are shown in Fig. 6. Added TTP and CTP produce the same CD changes. The spectra have a peak at 220 nm but, at the higher histone concentration, where there is more total interaction, the shoulder at 210 nm, found in α-helical difference spectra, is less pronounced.

**AMP and cAMP**

We note that we could detect no change upon the addition of AMP or cAMP in concentrations found effective for di- or triphosphates.

**DISCUSSION**

As has been pointed out (1, 2), the techniques of fluorescence anisotropy and CD, as used here, yield data that are functions of the changes in conformation of the protein. They do not measure the fraction of small molecules bound to histone IV, although the effective binding constants, as we have defined them, are useful in comparing the effectiveness of small molecules in inducing change. However, we emphasize that because of the nature of the data we cannot interpret them as true binding constants except in special cases, such as that in which only one molecule binds per histone. We can, nevertheless, draw some important conclusions.

(i) The binding of ATP or GTP to histone IV is very strong. Simple equilibrium calculations for one binding site per molecule show that an equilibrium constant of at least 10⁶ M⁻¹ is required to produce fractional binding at H₀ = 4.4 μM and ATP/H₀ = 2.0, which is within 5% of that found at H₀ = 17.5 μM and ATP/H₀ = 2.0. The value 5% is about the experimental error of the anisotropy measurement in Fig. 2.

(ii) Either the ATP binding is itself co-operative or the conformational change is sequential, with the binding of an ATP molecule or molecules producing one conformational change, while further binding produces a greater change. Either of these requires at least 2 binding sites.

(iii) There is both phosphate and nucleotide specificity. Triphosphate is stronger than diphosphate, which is stronger than monophosphate. ATP or GTP is much more effective than CTP or TTP. AMP and cAMP had no effect over the range tested.

The divergence of the CD curves at different histone concentrations (Fig. 4), in contrast to the fluorescence results of Fig. 2, could arise in several ways. The binding that produces the CD change could be at a different site than that which produces the fluorescence change, and there have a lower equilibrium constant. Alternatively, there could be some additional secondary structural formation at the higher histone concentration to which the anisotropy is insensitive. Interchain β-sheet would be a possible candidate for such a structure. If it occurred in the N-terminal end of the molecule the anisotropy might not be as sensitive to it as the CD, since the four tyrosines are all in the C-terminal half of the molecule (3, 4).

Although CTP and TTP are much less effective than ATP and GTP, the effective equilibrium constants obtained from the data in Fig. 5 are 10 times higher than those previously found for sulfate, which in turn is the most effective inorganic divalent anion tested (2). It should be pointed out that the effective equilibrium constant, 1.3 × 10⁴ M⁻¹, is the true equilibrium constant for the CTP- or TTP-histone IV binding if, but only if, there is only one binding site that affects the CD or fluorescence anisotropy. In that case, it is the binding constant for that site.

It is impossible to postulate any specific biological function for the interaction of nucleoside triphosphates with histone IV, and indeed there may be none. However, the possibility that such biologically important molecules exert controls on histone conformation is intriguing.

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