Interactions of the Nucleosomal Core Histones: A Calorimetric Study of Octamer Assembly†

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ABSTRACT: Enthalpies of assembly for histone subunits into the nucleosomal core octamer have been studied by isothermal heatburst microcalorimetry. The assembly follows a "mixed association" reaction scheme

$$D + T \rightleftharpoons DT \text{ (hexamer)} \quad (1a)$$

$$DT + D \rightleftharpoons D_2T \text{ (octamer)} \quad (1b)$$

where D is the H2A–H2B dimer and T is the $(H3–H4)_2$ tetramer [Eickbush, T. H., & Moudrianakis, E. N. (1978) *Biochemistry 17*, 4955]. The standard enthalpies for reactions 1a and 1b are found to be large and unequal at pH 7.5, 2 M NaCl, 25 °C ($\Delta H°_1 = -30.8$ kcal; $\Delta H°_2 = -22.5$ kcal). The determined equilibrium constants are in agreement with previous results that indicate cooperativity in the assembly process, the intrinsic equilibrium constants for reaction 1b being about 4 times as great as that of reaction 1a [Godfrey, J. E., Eickbush, T. H., & Moudrianakis, E. N. (1980) *Biochemistry 19*, 1339]. The calorimetric heats we have determined in different buffers indicate a small net proton release for overall octamer assembly. Direct titration shows the proton release to be approximately 0.2 proton per octamer formed at pH 7.5. This number increases as the pH is lowered. The bearing of these results on the types of dominant interactions stabilizing the octamer is discussed.

The elementary subunit of chromatin structure, the nucleosome (Thomas & Kornberg, 1975), contains a central octameric "core" of histones around which the nuclear DNA is wound [cf. McGhee & Felsenfeld (1980) for a recent review]. This fundamental complex is believed to play a central organizing role in the compaction of DNA and, at some level, in the regulation of gene expression. Understanding the exact biological functioning of this complex system of macromolecules will require information regarding the nature of interactions between its components and of how these interactions change during the functional cycle. In this paper we present results of a calorimetric study on subunit assembly of the core histone octamer from its fundamental dimeric and tetrameric subcomplexes (Eickbush & Moudrianakis, 1978; Godfrey et al., 1980).

Our studies of subunit assembly in this system are aimed toward understanding the nature of the interactions that account for the stability of the nucleosomal core particles. Subunit dissociation is a powerful means of probing the intersubunit contact regions of protein quaternary structures (Ackers, 1980; Godfrey et al., 1980). Additional interest stems from the recent implication that subunit dissociation may occur in vivo (Jackson & Chalkley, 1981). We present here the first calorimetric study on subunit assembly in this important protein system. Results of thermodynamic studies, in conjunction with crystallographic studies in progress (Lattman et al., 1982; Moudrianakis et al., 1982), will allow correlations between structural and energetic properties of the histone complex. Such information will be important in resolving the in vivo function of histones. The work reported here is made possible by a new method of obtaining large quantities of pure H2A–H2B and $(H3–H4)_2$ complexes under mild conditions (see Materials and Methods).

Studies in high-salt solutions have established that an octamer of the four core histones (H2A, H2B, H3, and H4) is formed in the absence of DNA (Thomas & Kornberg, 1975; Eickbush & Moudrianakis, 1978) and that a histone octamer of the same composition is the central organizing unit of chromatin (McGhee & Felsenfeld, 1980). Eickbush & Moudrianakis (1978) demonstrated the reaction to be a "mixed association" of two H2A–H2B dimers with an $(H3–H4)_2$ tetramer to form an intermediate hexamer, and, finally, the octamer.

$$\begin{aligned} D + T &\overset{K_1}{\rightleftharpoons} DT \text{ (hexamer)} \\ DT + D &\overset{K_2}{\rightleftharpoons} D_2T \text{ (octamer)} \end{aligned} \quad (1)$$

In this reaction scheme D represents the dimer, T the tetramer, DT the hexamer, and D_2T the octamer. $K_1 = (DT)/[(D)(T)]$ and $K_2 = (D_2T)/[(DT)(D)]$ where the quantities in parentheses are species concentrations. Strong dependencies of these reactions on salt, pH, and temperature were noted. Godfrey et al. (1980) confirmed the stoichiometry using equilibrium sedimentation, established that the reactions are reversible, and obtained equilibrium constants for the two reaction steps. Values of the derived equilibrium constants, K_1 and K_2, suggested cooperativity in the successive reaction steps; i.e., binding of the first dimer to the tetramer enhances binding of the second dimer by a factor of approximately four. The basis of this cooperative interaction is not known.

The marked pH dependence of the subunit association reactions, observed by Eickbush & Moudrianakis (1978), suggests the possibility that proton release, or adsorption, might be linked to protein–protein interactions as is the case in the subunit assembly in human hemoglobin (Chu & Ackers, 1981). Possible sources of cooperativity include alteration of contacts between the two H2A–H2B dimers within the octamers, or conformational changes of the $(H3–H4)_2$ tetramer, conveying energy from one dimer binding site to the other. The conformations of the histones are well-known to be sensitive to salts and small organic molecules (D'Anna & Isenberg, 1974a,b, 1972; Beaudette et al., 1982).

A large temperature dependence of the equilibria also noted by Eickbush & Moudrianakis (1978) suggests large enthalpies for the reactions. For this reason, we chose microcalorimetry

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as a means of probing this system. A "mixed associating system", such as shown in scheme 1, is a good candidate for study by isothermal, mixing microcalorimetry because of the experimental control afforded when the two different types of subunits can be manipulated independently (Valdes & Ackers, 1977).

Theory of the Calorimetric Determinations

In the calorimetric technique we employed, a solution containing dimers is mixed in the calorimeter with a second solution containing tetramers and the resulting heat Q_t is measured. The pertinent relationships between this calorimetric heat effect and the parameters of reaction scheme 1 that we wished to resolve are summarized below.

As a result of the calorimetric mixing experiment the species DT is formed in a certain concentration (DT), moles per liter. The heat produced by forming this species in a volume V of final solution is (DT) × ΔH_1 × V, where ΔH_1 is the molar enthalpy of formation for tetramers with one dimer bound. Similarly, a certain number of moles of species D_2T will be formed with heat effect (D_2T) × ΔH_T × V, where ΔH_T is the total enthalpy over both reaction steps of scheme 1, i.e., $\Delta H_T = \Delta H_1 + \Delta H_2$ where ΔH_2 is the enthalpy of the second reaction. The total heat Q_t divided by the total number of moles of tetramer in solution will be

$$\frac{Q_t}{(T_t)} = \frac{(DT)}{(T_t)}\Delta H_1 + \frac{(D_2T)}{(T_t)}(\Delta H_1 + \Delta H_2) \quad (2)$$

$$\frac{Q_t}{(T_t)} = \left[\frac{(D_t)}{(T_t)} - \frac{(D)}{(T_t)}\right]\Delta H_1 + \frac{(D_2T)}{(T_t)}[\Delta H_2 - \Delta H_1] \quad (3)$$

where $(D_t) = (D) + (DT) + 2(D_2T)$ and $(T_t) = (T) + (DT) + (D_2T)$. The total concentrations (D_t) and (T_t) are predetermined in the calorimetric experiment. These relationships and the definitions of the equilibrium constants of reaction scheme 1 provide a system of equations in the four unknowns K_1, K_2, ΔH_1, and ΔH_2. These parameters can be resolved from a series of experiments where both the total concentration and their ratios $(D_t)/(T_t)$ are independently manipulated and the resulting values of Q_t determined.

The effects of proton uptake or release that may accompany these reactions can be incorporated into the analysis by carrying out experiments in a series of buffers having different heats of ionization. For each of the enthalpies, say ΔH_1, a relationship can be written

$$\Delta H_{1,app} = \Delta H_1 + \Delta n_1 \Delta H_{buff} \quad (4)$$

where ΔH_1 is the component of enthalpy exclusive of ionization heats, Δn_1 is the number of protons taken up in the protein assembly reaction, and ΔH_{buff} is the heat of ionization of the buffer components in which the reaction is carried out. If experiments are done with a series of buffers having known values of ΔH_{buff}, the resulting data can be used to resolve ΔH_1 and Δn_1 and similar terms for each reaction. Thus, in the studies reported here, there are altogether six parameters of interest: K_1, K_2, ΔH_1, ΔH_2, Δn_1, and Δn_2.

Materials and Methods

Protein Samples. Calf thymus chromatin was isolated essentially as described earlier (Eickbush & Moudrianakis, 1978). Homogeneous preparations of histone octamers and its subunits, i.e., the H2A–H2B dimer and the $(H3–H4)_2$ tetramer, were prepared by a new column chromatographic fractionation procedure to be described in detail elsewhere (R. C. Benedict and J. E. Godfrey, unpublished experiments). One of the advantages of this new method over earlier histone fractionation procedures is that it yields the histone dimer and tetramer subunits virtually free of cross contamination, and this is particularly important in the case of the histone H2A–H2B dimer. The state of purity as well as polypeptide integrity was monitored by SDS–urea–polyacrylamide gel electrophoresis (Eickbush & Moudrianakis, 1978). No proteolytic degradation was detected for samples stored at 5 °C for up to 10 days. All samples were dialyzed exhaustively into the experimental buffer and used within 10 days. Concentrations were determined by using extinction coefficients ($A^{277nm}_{1mg/mL}$ = 0.484, dimer; $A^{277nm}_{1mg/mL}$ = 0.444, tetramer) along with the method of scattering correction described by R. C. Benedict and J. E. Godfrey (unpublished experiments). Scattering corrections were always less than 5%.

Table I: Solution Conditions for the Calorimetric Mixing Experiments

data set no.	buffer	(dimer) (mg/mL)	(tetramer) (mg/mL)
1	2 M NaCl, 0.1 M Tris, 1 mM EDTA	2.34	1.71
2	2 M NaCl, 0.1 M PIPES, 1 mM EDTA	6.83	6.61
3	2 M NaCl, 0.1 M HEPES, 1 mM EDTA	7.00	5.61
4	1 M NaCl, 0.1 M Tris, 1 mM EDTA	5.74	5.09

Calorimetry. All calorimetric data were obtained with an LKB isothermal batch heat-burst microcalorimeter (Model 10700). Data were collected at 25 ± 0.5 °C. Instrument performance was checked by reproduction of glycerol dilution heats (Goudard & Leydet, 1965). The uncertainty of each measurement is estimated to be ±0.1 mcal. Experiments were performed by forming a series of reaction solutions that were then mixed in various ratios $(D_t)/(T_t)$ to generate a range of heat effects. Each pair of reaction solutions was in a different buffer system for which the heat of ionization was also redetermined in this study. Compositions of reaction solutions reported in this paper are given in Table I.

Analysis of Data. Nonlinear least-squares analysis of data (Turner et al., 1981) was performed on a Hewlett-Packard HP-1000 computer. Uncertainties and weighting factors were assigned as described by Bevington (1969). Error limits were assigned to a given parameter by fixing the parameter at values different from the least-squares minimum and using the fitting program to search for a best fit by varying the remaining parameters of the model. In this procedure the value of χ^2 is always higher than the best fit value, and a particular ratio of the test χ^2 value to the best fit χ^2 value is characteristic of a confidence contour in the parameter space. The F statistic was used to define the χ^2 ratio associated with an approximate 75% confidence contour (Draper & Smith, 1966). No local minima were encountered either in the assignment of error extremes or in the searching for best fit parameters.

Protein Titration. Dimeric and tetrameric solutions were dialyzed against 2 M NaCl held to the approximate pH with phosphocellulose, an undialyzable buffer. The protein solutions were adjusted to the final pH after dialysis. An aliquot of the tetramer solution was placed in a jacketed beaker thermostated to 25 °C and equipped with a stirrer (TTA80 titration assembly, Radiometer, Copenhagen). The pH was monitored with a combination electrode (GK2421C, Radiometer, Copenhagen). The dimer solution was added in steps with a calibrated glass syringe with micrometer drive. At each step the protein solution was restored to the original pH by addition

Table II: Evaluation of Thermodynamic Constants

deg of freedom		variance of fit[a]	$K_{1,int}$[b] (M^{-1})	$K_{2,int}$ (M^{-1})	ΔH_1 (kcal/mol)	ΔH_2 (kcal/mol)	Δn_1	Δn_2	ΔH_T[c] (kcal/mol)	Δn_t
13		2.68×10^{-5}	2.12×10^5	9.96×10^5	−30.8	−22.5	0.25	−0.35	−53.3	−0.10
75% confidence error limits:	upper limit				−27.7	−19.7	0.68	0.05	−47.4	0.73
	lower limit				−33.9	−25.3	−0.20	−0.75	−59.2	−0.95

[a] The results shown are for the fit with two equilibrium constants, two enthalpies, and two proton release terms varied. Each of the 19 data points was weighted, as described in the text. [b] The results of Godfrey et al. (1980), corrected to 25 °C, yield intrinsic equilibrium constants of $K_1 = 1.69 \times 10^5$ M^{-1} and $K_2 = 8.58 \times 10^5$ M^{-1}. [c] Total enthalpies and proton release values were calculated from the best fit parameters.

Table III: Analysis of the Data in Terms of Models Requiring Equal Enthalpies or Zero Proton Release[a]

deg of freedom	variance of fit	K_1[b] (M^{-1})	K_2 (M^{-1})	ΔH_1[c] (kcal/mol)	ΔH_2 (kcal/mol)	Δn_1[b]	Δn_2	ΔH_T[d] (kcal/mol)	Δn_t
16	4.42×10^{-5}	(4.23×10^5)	(4.98×10^5)	[−27.0]	[−27.0]	0.68	−0.88	−54.0	−0.20
14	4.68×10^{-5}	1.51×10^6	4.39×10^5	[−27.0]	[−27.0]	0.35	−0.55	−54.0	−0.20
17	2.78×10^{-5}	(4.23×10^5)	(4.98×10^5)	−32.0	−20.7	(0)	(0)	−52.7	(0)
15	2.81×10^{-5}	7.81×10^5	3.38×10^5	−30.4	−22.7	(0)	(0)	−53.1	(0)

[a] Each data point was assigned a weight, as described in the text. [b] Equilibrium constants and proton release terms in parentheses were held fixed at the values shown. [c] Enthalpy terms shown in brackets were forced to be equal during the fitting. [d] Total enthalpies and proton releases were calculated from the best fit parameters.

of carbonate-free sodium hydroxide (1 mM) in 2 M NaCl. This pH-stat experiment directly yields the proton release that accompanies the binding of dimer to tetramer. Problems arising from inexact matching of the protein solution pHs and slow electrode drift were corrected for by base-line subtraction. Above the stoichiometric dimer to tetramer ratio of 2 the curves should level off, yielding the total proton release. This criterium was used for selecting a base line of constant slope.

Results

Large heat releases (i.e., 1–8 mcal) were observed upon mixing solutions of dimer with tetramer. The heat release profiles showed no evidence of any slow processes and were similar to profiles generated by a 20-s electrical burst to the calibration heater of the calorimeter. The measurements may thus be presumed to accurately reflect the heat release corresponding to a final state of equilibrium. Reversibility of the octamer formation was also found in equilibrium ultracentrifugation measurements (Godfrey et al., 1980). Data obtained in 2 M NaCl were highly reproducible.

Calorimetric mixing data were collected in three high-salt buffers: (a) 2 M NaCl, 0.1 M Tris, 1 mM EDTA, pH 7.5; (b) 2 M NaCl, 0.1 M PIPES, 1 mM EDTA, pH 7.5; (c) 2 M NaCl, 0.1 M HEPES, 1 mM EDTA, pH 7.5. An additional data set was collected in 1 M NaCl–0.1 M Tris–1 mM EDTA, pH 7.5. The reported enthalpies of protonation of these buffers at low ionic strengths are −12.2 kcal/mol (Tris), −3.3 kcal/mol (PIPES), and −5.5 kcal/mol (HEPES) (Good et al., 1966). In order to evaluate possible effects of the high ionic strength on these protonation heats, we redetermined them by separate calorimetric measurements. The buffer protonation heats listed above were found to be the same in the 2 M NaCl buffers by these measurements.

The high-salt data for all the buffers are presented in Figure 1 where the enthalpy values $Q_t/(T_t)$ are plotted against the ratios $(D_t)/(T_t)$. The solid lines are drawn according to limiting forms of eq 2 or 3, as follows. When saturating ratios of dimer to tetramer exist, the species T and DT vanish and $(D_2T) = (T_t)$. Hence, eq 2 reduces to

$$\frac{Q_t}{(T_t)} = \Delta H_1 + \Delta H_2 \qquad (5)$$

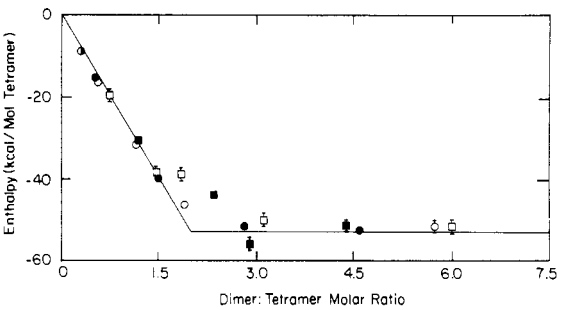

FIGURE 1: Heats producted upon reaction of histone dimers with tetramers at 25 °C, pH 7.5. (□) 2 M NaCl, 0.1 M Tris, 1 mM EDTA, data set 1; (●) 2 M NaCl, 0.1 M PIPES, 1 mM EDTA, data set 2; (■) 2 M NaCl, 0.1 M HEPES, 1 mm EDTA, data set 3; (○) 2 M NaCl, 0.1 M Tris, 1 mM EDTA, data set 4. Enthalpies of reaction are the measured heats Q_t, normalized with respect to the total concentration (T_t) of tetramer in the reaction vessel. Solid lines are theoretical asymptotes for eq 3, drawn for a total enthalpy of −53 kcal/mol for the two reactions (see the text). High protein concentration or association constants would push the data toward these lines. The intersection of the line segments at a dimer to tetramer ratio of 2 indicates the stoichiometry of the reaction.

The horizontal solid line is a plot of this limiting relationship using values of ΔH_1 and ΔH_2 determined from analysis of the composite of all data points shown. The sloping line of Figure 1 is a plot of

$$\frac{Q_t}{(T_t)} = \frac{1}{2}\Delta H_t \frac{(D_t)}{(T_t)} \qquad (6)$$

approximately the situation when dimer to tetramer ratios are not saturating but equilibrium constants K_1 and K_2 are sufficiently large, so that all dimers added to the system react with tetramers.

Evaluation of Thermodynamic Parameters. In the least-squares fitting, the high-salt calorimetric data were well-described by the two-step binding of dimer to tetramer, as shown in reaction scheme 1. These equilibria are characterized by equilibrium constants, K_1 and K_2, two corresponding enthalpies, ΔH_1 and ΔH_2, and two proton release terms, Δn_1 and Δn_2. The weighted data were fit by optimizing these six parameters in various combinations, as shown in Tables II and III. The two equilibrium constants found when all parameters were evaluated simultaneously (Table II) are very close to the values

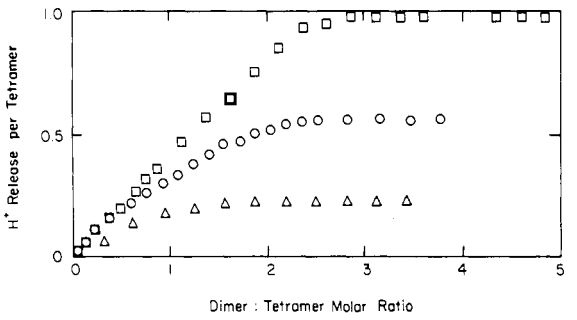

FIGURE 2: Results of protein titration experiments. Proton release is plotted against dimer to tetramer molar ratio. All in 2 M NaCl, 25 °C. Conditions: (□) pH 6.7, 8.74 mg/mL dimer, 7.52 mg/mL tetramer; (○) pH 7.2, 7.07 mg/mL dimer, 4.77 mg/mL tetramer; (△) pH 7.5, 8.45 mg/mL dimer, 6.44 mg/mL tetramer.

of Godfrey et al. (1980) when the latter are corrected to 25 °C by using the enthalpies determined in this study. Although the equilibrium constants are not well-defined by the calorimetric data, due to high cross correlation between parameters [cf. Turner et al. (1980)], the difference found for the successive enthalpies was insensitive to the exact values of equilibrium constants.

Values of the two reaction enthalpies reported in Table II show a large difference (8.3 kcal/mol). The confidence limits (Table II) on these estimated values do not overlap, and it is unlikely that the difference between them arises from experimental uncertainty. The estimated value for the total proton uptake over the two reaction steps is 0.1 mol of ΔH^+/mol of octamer formed. This value is consistent within experimental error, with the value (0.2 proton) estimated from the titration data at pH 7.5 (see below). Compensating proton releases are predicted in the best fit, i.e., 0.25 proton released in the first step and 0.35 proton absorbed in the second. At the 75% confidence level, however, equal numbers of proton release for the two reactions are also consistent with the data. In order to explore further the possible relationships between unequal enthalpies and proton binding effects, the data were fit by using several constraints (Table III). (1) It was found that forcing ΔH_1 and ΔH_2 to be equal provided a poorer fit of the data and resulted in prediction of large proton releases for the overall reaction. Allowing the equilibrium constants to be adjusted freely did not alter these results. (2) Also, it was found that fixing the proton release terms to zero and allowing ΔH_1 and ΔH_2 to be optimized returned a good fit to the data and essentially the same values for these two heats as from the full fit of the data. Very similar results are obtained if the equilibrium constants and enthalpies are adjusted simultaneously. A summary of these analyses is presented in Table III. The data are best represented by the combination of unequal enthalpies and no net proton release. The variance of the fit in this case is significantly better than for the case requiring equal enthalpies. This conclusion is not affected by small changes in the equilibrium constants, as shown by Table III.

Proton Titration. The suggestion of a proton release from the calorimetric data prompted a titration experiment that was carried out to measure this effect directly. The proton release induced by mixing different amounts of the H2A–H2B and $(H3–H4)_2$ complexes was determined. In Figure 2 the net proton release is plotted against the molar ratio of dimer to tetramer at three different pHs. These experiments, conducted in 2 M NaCl at 25 °C, show a net proton release at pH 7.5 of about 0.2 mol of H^+/mol of octamer formed. This is within the experimental error of the calorimetric analysis. The data shown at pH 7.2 and 6.7 show that the total release increased to 0.6 and 1.0, respectively.

Table IV: Thermodynamic Parameters for Histone Assembly Reactions

$\Delta G^\circ_{1,int}$ (kcal/mol)	−7.3
$\Delta G^\circ_{2,int}$ (kcal/mol)	−8.2
ΔH_1 (kcal/mol)	−30.8
ΔH_2 (kcal/mol)	−22.5
$\Delta S^\circ_{1,int}$ (cal mol^{-1} K^{-1})	−76
$\Delta S^\circ_{2,int}$ (cal mol^{-1} K^{-1})	−48
$\Delta G^\circ_{2,int} - \Delta G^\circ_{1,int}$ (kcal/mol)	−0.9
$\Delta H_2 - \Delta H_1$ (kcal/mol)	8.3
$\Delta S^\circ_{2,int} - \Delta S^\circ_{1,int}$ (cal mol^{-1} K^{-1})	28

Solution Effects. The high-salt (2 M NaCl) data plotted in Figure 1 are highly reproducible and independent of the buffer used. For a data set obtained in 1 M NaCl–0.1 M Tris, pH 7.5, the estimated total enthalpy was found to be significantly lower. This effect is currently being explored. Large effects of salt have been reported on histone conformation (D'Anna & Isenberg, 1974a,b, 1972) and association (Eickbush & Moudrianakis, 1978).

There is no evidence of any effect attributable to the three different buffers used in this study; i.e., the data are well explained without the necessity of invoking effects of buffer binding. It should be pointed out, however, that histones stored in the 2 M KCl–0.1 M phosphate–1 mM EDTA, pH 7.5, buffer system were used within the first week following their isolation, since storing them at 5 °C in this buffer for long times yielded a small amount of precipitate. This precipitation did not involve proteolysis and is thought to reflect a conformational change induced by phosphate. Phosphate has previously been shown to be effective in causing conformational changes of these proteins (D'Anna & Isenberg, 1974a,b). Because of the presence of phosphate groups in DNA, it is possible that this change is of physiological importance. More thorough studies of these effects are currently under way.

Discussion

This study offers the first thermodynamic examination of the energetics of assembly in the histone octamer. The results presented show that the two steps of octamer formation in high salt are accompanied by large unequal enthalpies and a concomitant proton release. Complete analysis of this system will require correlation of high-salt data with experiments performed on the protein–DNA complex, more thorough information on biological function than is currently available, and of course the crystallographic structure determination. Table IV summarizes the thermodynamic parameters established in the present study.

The results of this work provide additional evidence that the two reaction steps of dimer with tetramer are not equal. We find cooperativity in the equilibrium constants ($K_{1,int}$ = 2.12 × 10^5 M^{-1}; $K_{2,int}$ = 9.96 × 10^5 M^{-1}) after correction for statistical factors, in agreement with the earlier results of Godfrey et al. (1980). The calorimetric data show a difference of approximaately 8.3 kcal/mol in the heat release from the two assembly steps. A better understanding of the interdependence between the unequal enthalpic effects and proton binding reactions will be necessary for the analysis of nucleosome core particle stability. The large heats found in this study for the protein–protein interactions, in particular, might have a bearing on the interpretation of previous calorimetric studies of the denaturation of nucleosomes (Bina et al., 1980) and nucleosomal core particles (Weischet et al., 1978). The large exothermic heats associated with the histone assembly reactions are consistent with a dominance of H bonding (Ackers, 1980; Ross & Subramanian, 1981) as previously suggested by

Eickbush & Moudrianakis (1978).

Several types of processes can be ruled out as dominant factors in the energetics: A difference in ion pairing in an aqueous environment would not generate the large enthalpy observed (Ross & Subramanian, 1981; Ross & Shapiro, 1974). A change in the aqueous ionic shielding atmosphere of the charged residues would also be a largely entropic effect (Pfeil & Privalov, 1976). Hydrophobic interactions are entropy driven (Gill & Wadso, 1976) and not a good candidate for the dominant source of the energetic effects.

The proton release results suggest that the unprotonated form of a group or groups with pK_a of about 6.7 may be important in stabilizing the octamer. Cysteine and histidine residues in proteins titrate in this range, and both are present in the core histone. The only strong interaction known between isolated histones of the dimer and histones of the tetramer is between H2B and H4 (Isenberg, 1979). The C-terminal ends of these proteins have been shown by cross-linking studies to interact (Delange et al., 1979; Martinson et al., 1979). Histidines-82 and -109 of H2B are within the regions known to be important in this interaction (McGhee & Felsenfeld, 1980). One or both of these are likely prospects for the pH sensitivity effect.

We believe that such interactions endow the system with properties uniquely suited for delicate biological regulation. The histone octamer is a tripartite structure with two internal contact interfaces symmetrically situated on either side of the centrally located tetramer (Eickbush & Moudrianakis, 1978). We envision these interfaces to lie more or less normal to the axis of the DNA supercoil formed by the wrapping of the double helix around the compact histone octamer (Moudrianakis et al., 1977) to yield the nucleosome (Klug et al., 1980). The involvement of a limited number of H bonds in the dimer–tetramer assembly offers a high degree of specificity in the contact of these interfaces while also keeping the energetic requirements of the reversibility of these processes to a minimum. We have suggested earlier (Moudrianakis et al., 1977, 1982) that the controlled opening and closing of these interfaces might lead to the reversible compaction of the DNA and thus serve as the regulatory basis for the transition between active and inert states of the genetic material. The findings of the present study provide a more quantitative basis for the eventual understanding of these regulatory processes.

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