Effects of Ionic Strength and State of Assembly on Kinetics of Hydrogen Exchange of Calf Thymus Histones†

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ABSTRACT: The kinetics of hydrogen exchange of calf thymus histone H2A–H2B dimers and $(H3–H4)_2$ tetramers at pH 7 have been examined at low (0.16 M NaCl) and high (2 M NaCl) ionic strengths and after incorporation into $(H2A–H2B–H3–H4)_2$ octamers. The similarity of the results for both species is noteworthy. Approximately 60% of the backbone amide protons are detectable in both low and high salt, and at least three kinetic phases can be distinguished. Increasing the ionic strength from 0.16 to 2 M accelerates exchange of some of the rapidly exchanging protons in both dimers and tetramers, while slowing exchange of others. Exchange of the more slowly exchanging protons is virtually unaffected. Incorporation of dimers into octamers accelerates exchange of ~40 protons to such an extent that they can no longer be detected. The effects of assembly upon the tetramer are qualitatively similar. These results indicate that both high ionic strengths and assembly destabilize some regions of the structure while stabilizing others. For both dimers and tetramers, the effects of ionic strength are dramatic, while those of assembly are more subtle. Higher resolution studies aimed at identifying the responsive protons would be of interest.

The fundamental subunit of chromatin structure, the nucleosomal core particle, consists of a histone octamer, containing 2 mol each of histones H2A, H2B, H3, and H4, around which 1.5 turns of DNA are wrapped [cf. McGhee & Felsenfeld (1980) or Sperling & Wachtel (1982) for recent reviews]. Each octamer is in turn composed of a $(H3–H4)_2$ tetramer which appears to be sandwiched between two H2A–H2B dimers. In the absence of DNA, the octamer is stable at high ionic strengths but dissociates at low ionic strengths to form $(H3–H4)_2$ tetramers and H2A–H2B dimers [cf. Eickbush & Moudriankis (1978)]. This equilibrium may be significant in vivo since recent evidence indicates that newly synthesized $(H3–H4)_2$ tetramers associate with newly synthesized DNA well in advance of newly synthesized H2A–H2B dimers (Jackson & Chalkley, 1981).

Several chemical and spectroscopic probes have been used to examine the effects of ionic strength and assembly on the time-averaged structures of the nucleosomal core histones [cf. Weintraub et al. (1975), Thomas et al. (1977), Cotter & Lilly (1977), Fulmer & Fasman (1979), Godfrey et al. (1981), and Beaudette et al. (1981)]. The effects of DNA–histone interactions upon the structural dynamics of the DNA have also been examined and shown to be negligible (Mitane et al., 1980). The dynamics of the histones have, however, been examined only on the NMR time scale (Bradbury, 1983), despite increasing interest in the physical and functional significance of molecular fluctuations in proteins. [For recent reviews, see Gurd & Rothgeb (1979) and Karplus & McCammon (1982).] In this study, we have examined the kinetics of hydrogen exchange from calf thymus histone dimers and tetramers. We find that changes in ionic strength have profound effects on the kinetics of exchange of both species. Similar, although more subtle, changes accompany assembly. By carrying these studies to higher resolution, it should be possible to obtain new information about those regions of the structure which are involved in, or perturbed by, subunit interactions.

Materials and Methods

Proteins were prepared as described by Benedict et al. (1984) from frozen calf thymus stored at −30 °C. Purified proteins were stored at 4 °C in 2.0 M NaCl, 20 mM sodium *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonate (Na^+-Hepes), and 1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.0, and used within 3 weeks. No degradation was detected by sodium dodecyl sulfate (SDS)–urea–polyacrylamide electrophoresis. Concentrations were determined spectrophotometrically; values of $E^{1\%,\,1\,cm}_{277\,nm}$ of 4.84, 4.44, and 4.64 and molecular weights of 28 000, 53 000, and 109 000 were assumed for H2A–H2B, $(H3–H4)_2$, and $(H2A–H2B–H3–H4)_2$, respectively (Benedict et al., 1984).

Hydrogen exchange experiments were performed as described by Lennick & Allewell (1981). The two-column gel chromatographic methods of Englander & Englander (1972) were used for solvent separation. Following Schreier (1977), trace amounts (<5%) of protein labeled with $H^{14}CHO$ and reduced with $NaBH_3CN$ (Jentoft & Dearborn, 1979) were used as an internal concentration standard. Since histones precipitate under the conditions required for labeling, the labeled protein was either ovalbumin (Sigma) or the catalytic subunit of *Escherichia coli* aspartate transcarbamylase. While differential elution of tracer and histones could in principle be a problem, the fact that very similar results were obtained regardless of the protein used as tracer indicates that this is unlikely to be the case. Aqualyte Plus (Baker) was the scintillation fluid. The procedures described by Kobayashi & Maudsley (1974) were used to correct for spillover between channels.

Exchange in was accomplished by equilibrating protein, at concentrations of 1–3 mg/mL, with ^3H_2O (20 mCi/mL) in 2 M NaCl, 20 mM Hepes, and 1 mM EDTA at pH 7 and 25 °C for 18 h. Exchange out was carried out at 0 °C either in

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Table I: Values of Parameters Derived by Fitting Data to Equation 1

expt	N_1	k_1 (h^{-1})	N_2	k_2 (h^{-1})
dimer, low salt	119 ± 22	1.0 ± 0.3	29 ± 24	0.1 ± 0.1
dimer, high salt	43 ± 14	7.7 ± 4.0	94 ± 7	0.08 ± 0.02
dimer in octamer, high salt	0	0	89 ± 7	0.08 ± 0.03
tetramer, low salt	190 ± 43	2.3 ± 1.2	89 ± 57	0.19 ± 0.16
tetramer, high salt	108 ± 37	10 ± 7	178 ± 15	0.08 ± 0.03
tetramer in octamer, high salt	39 ± 37	6.4 ± 11	173 ± 12	0.03 ± 0.02
octamer, high salt	106 ± 56	8 ± 7	296 ± 10	0.06 ± 0.01
octamer (dissociated), low salt	267 ± 59	0.82 ± 0.45	160 ± 18	0.09 ± 0.04

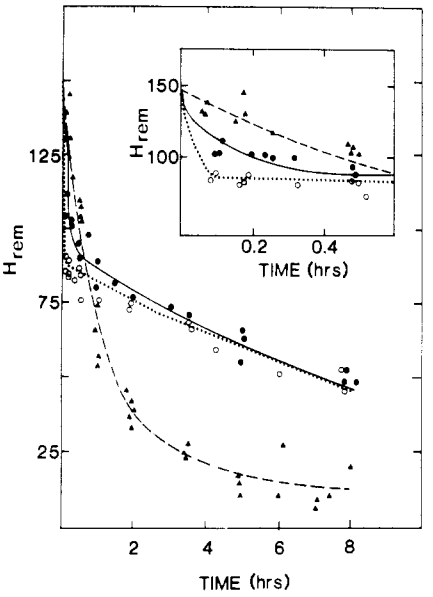

FIGURE 1: Kinetics of exchange out from H2A–H2B at pH 7, 0 °C, under high salt conditions (2 M NaCl, 20 mM Hepes, and 1 mM EDTA) and low salt conditions (0.16 M NaCl, 20 mM Hepes, and 1 mM EDTA) and after incorporation into $(H2A–H2B–H3–H4)_2$. In all cases, exchange in was accomplished by equilibration with ^3H_2O (20 mCi/mL) in 2 M NaCl, 20 mM Hepes, and 1 mM EDTA at pH 7 and 25 °C for 18 h. (●, —) High salt; (▲, - - -) low salt; (○, ···) dimer in octamer. Insert: Initial kinetics of exchange, expanded scale.

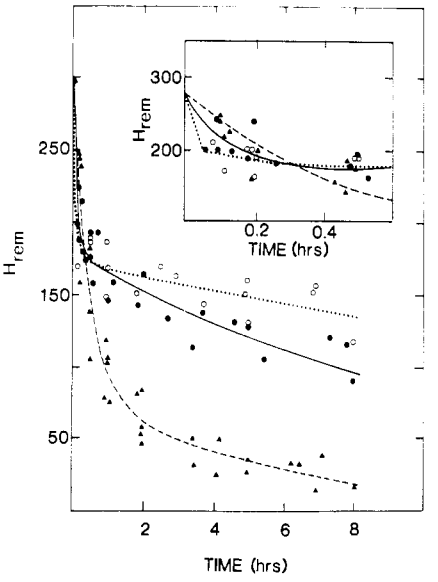

FIGURE 2: Kinetics of exchange out from $(H3–H4)_2$ at pH 7, 0 °C, under high salt conditions (●, —) and low salt conditions (▲, - - -) and after incorporation into octamers (○, ···). Insert: Initial kinetics of exchange, expanded scale. Experimental conditions are as described in Figure 1.

the same buffer (high salt conditions) or in 0.16 M NaCl, 20 mM Hepes, and 1 mM EDTA, pH 7 (low salt conditions). In the latter case, the first column was equilibrated with low salt buffer. In reconstitution experiments, the protein solution eluted from the first column was collected into a 3–6-fold excess of the complementary subunit.

Complete reconstitution of $(H2A–H2B–H3–H4)_2$ from H2A–H2B and $(H3–H4)_2$ was demonstrated by small-zone analytical gel chromatography on a Sephadex G-150 column (30 × 0.9 cm) calibrated with dimers, tetramers, and octamers.

H_{rem}, the number of protons still unexchanged per molecule at time t, was computed as described by Englander & Englander (1972). A nonlinear least-squares program provided by Michael Johnson (University of Virginia, Charlottesville, VA) was used to fit the data to

$$H_{rem} = \sum_i N_i e^{-k_i t} \quad (1)$$

where N_i is the number of protons in the ith kinetic class, k_i is the rate constant for their exchange, and t is time.

Results

Exchange-out curves for H2A–H2B dimers are shown in Figure 1. At least two kinetic classes are readily distinguishable in both 0.16 and 2 M NaCl. Interestingly, incorporation of dimers into octamers appears to accelerate exchange from the first class to such an extent that their exchange is complete before the first time point (4–5 min).

The results of fitting these data to the sum of two exponentials (eq 1) are given in Table I. Although the rates of exchange undoubtedly span a wide range, no improvement in the fit was achieved by including more than two kinetic classes. The total number of protons whose rates of exchange fall within the detectable range is very similar under both high salt and low salt conditions. This number corresponds to ~57% of the backbone amide protons. The amount of label incorporated did not change significantly as a result of either increasing the time of labeling to 40 h or decreasing the temperature during exchange to 15 °C.

Shifting from low salt to high salt conditions appears to reduce the number of protons in the first class by approximately a factor of 3, while at the same time accelerating their rate of exchange by a factor of 7.7. Incorporation into the octamer increases the rate of exchange of the fast class so much that it becomes undetectable; however, the slow class appears unaffected.

The results for $(H3–H4)_2$ tetramers are remarkably similar (Figure 2). The total number of protons whose exchange can be detected under both high salt and low salt conditions is approximately 60% of the total number of backbone amide protons. Shifting from low salt to high salt conditions reduces the number of protons in the fast class but increases their rate of exchange. Since the magnitudes of both effects are smaller than those for dimers, exchange of some of the protons in the fast class can be detected. Incorporation into the octamer appears to produce an even greater decrease in the number of protons in the fast class; however, in contrast to the results

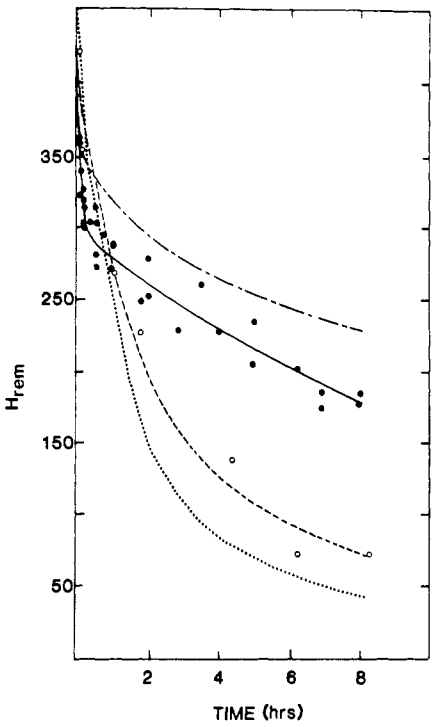

FIGURE 3: Kinetics of exchange out from octamers in 20 mM Hepes–1 mM EDTA, pH 7, 0 °C, under high salt (2 M NaCl) and low salt (0.16 M NaCl) conditions. Exchange-in conditions are as described in Figure 1. High salt (●, —); low salt (○, - - -). Octamers will be fully dissociated under low salt conditions. (– – –) Predicted exchange-out curve calculated from curves for H2A–H2B and $(H3–H4)_2$ incorporated into octamers in high salt in Figures 1 and 2. (···) Predicted exchange-out curve calculated from low salt data in Figures 1 and 2.

obtained with H2A–H2B, exchange from this class can be detected in the octamer.

Figure 3 shows the exchange kinetics of $(H2A–H2B–H3–H4)_2$ octamers under both high salt and low salt conditions. Dissociation into dimers and tetramers would be expected to be complete under low salt conditions (Godfrey et al., 1981). The curve for octamers in high salt falls slightly below the curve calculated from the data in Figures 1 and 2 for dimers and tetramers incorporated into octamers. This probably results from reductions in the numbers of protons in both the slow and fast classes because of less than complete labeling of the octamer. In contrast, the curve for the octamer under low salt conditions falls slightly above the weighted sum of the curves for the dimer and tetramer in low salt. This may reflect an increase in the number of detectable protons in the fast class, possibly because of the equilibrium between associated and dissociated species.

Discussion

Rates of hydrogen exchange from proteins depend upon solvent accessibility, with the slower rates reflecting the frequency of molecular fluctuations which transiently expose to solvent protons which are usually buried in the time-averaged structure. The biphasic nature of most of the exchange-out curves obtained in this study suggests that the exchangeable protons in both H2A–H2B dimers and $(H3–H4)_2$ tetramers fall into two major classes, each of which probably includes many subclasses. The two classes appear to interact in such a way that exchangeable protons shift from one class to the other with changing conditions.

Two domains of differing stability have been identified in both dimers and tetramers both by NMR measurements (Bradbury, 1983) and by limited proteolysis [cf. Hatch et al. (1983)]. These studies indicate that the amino terminus of H3 and H4 and both the amino and carboxyl termini of H2A and H2B are relatively unstructured. Susceptibility of the 10 carboxyl-terminal residues of H2A to trypsin is reduced to some extent when DNA binds and to a greater extent in octamers in high salt (Hatch et al., 1983). It is possible that the rapidly exchanging protons are located in amino- or carboxyl-terminal regions while the more slowly exchanging protons are in the trypsin-resistant core. This assignment does not, however, account for those protons which exchange too rapidly to be detected. Furthermore, if these regions are completely unstructured, exchange would in fact be expected to be even faster than observed. It is more likely, therefore, that all of the protons characterized in this study are located in relatively immobilized regions. The two classes may simply correspond to surface and buried protons. Alternatively, they may occur in two discrete but interacting domains. Measurements of net exchange rates from trypsin-treated preparations or medium resolution studies [cf. Rosa & Richards (1979)] will make it possible to distinguish these possibilities.

Since high ionic strengths promote the assembly of histone polypeptides into octamers which appear very similar to the octamers in core particles (D'Anna & Isenberg, 1972; Thomas et al., 1977; Cotter & Lilly, 1977; Eickbush & Moudrianakis, 1978; Beaudette et al., 1981; Godfrey et al., 1981), the effects of variations in ionic strength on the molecular dynamics of both subunits and octamer are of fundamental interest. We find for both dimers and tetramers that increasing ionic strengths reduce the number of protons in the rapidly exchanging class but accelerate their rate of exchange. Rates of exchange of the slow class are virtually unaffected. The reduced rate of exchange of some members of the fast class is not unexpected, since Kim & Baldwin (1982) found that increasing the ionic strength from 0.16 to 2 M slows exchange from poly(DL-lysine) by approximately a factor of 5. The increased rate of exchange of other protons indicates, however, that a portion of the structure is destabilized by high ionic strength.

The changes in the kinetics of exchange of both dimers and tetramers produced by assembly at high ionic strengths are subtle, suggesting that conformational differences between octamers and isolated dimers and tetramers depend primarily upon ionic strength, rather than protein–protein interactions. Incorporation into octamer does, however, appear to destabilize both dimers and tetramers to an even greater extent than high ionic strengths alone. Upon incorporation, all of the protons in the fast class of the dimer exchange too rapidly to be detected, while only 40 protons in the fast class of the tetramer can be detected. This loosening of the structure may be related to the cooperativity of assembly (Godfrey et al., 1981; Benedict et al., 1984). Again, rates of exchange from the slow classes in both subunits appear essentially unaltered. This is an unusual pattern; usually protein–protein interactions reduce rates of exchange, as a result of both shielding groups and damping vibrational modes [cf. Woodward & Ellis (1975), Schreier & Baldwin (1977), and Lennick & Allewell (1981)]. There are, however, several examples of binding of a small ligand accelerating exchange [cf. Malin & Englander (1980), Kiener & Waley (1977), Nonnenmacher et al. (1971), Printz & Gounaris (1972), and Reynolds et al. (1973)].

Since rates of exchange are sensitive to the amounts and stability of the secondary structure [cf. Englander et al. (1972) and Kossiakoff (1982)], it is of interest to compare our results with secondary structure predictions. Both H2A–H2B and $(H3–H4)_2$ are generally believed to contain significant amounts of α-helix and small amounts of β-structure. For example,

using protein basis spectra (Chen et al., 1970), Beaudette et al. (1981) estimated the α-helical content of dimers and tetramers to be 45% and 26%, respectively, at low ionic strength; 10% or less of both species appears to form β-structure. Increasing the ionic strength has a negligible effect on β-structure but increases the α-helical content of both dimers and tetramers to 45–50% (Beaudette et al., 1981).

Our results indicate that under low salt conditions 11% of the protons in H2A–H2B dimers and 19% of the protons in $(H3–H4)_2$ tetramers are in the slowly exchanging class. Under high salt conditions, these values increase to $\sim$34% for both dimer and tetramer. It appears likely then that the protons whose exchange kinetics are observable represent some, but not all, of the amide protons found in regions of secondary structure.

These preliminary results suggest that further analysis of the molecular dynamics of histones would be of considerable value. Areas of interest include the effects of covalent modification and DNA binding and the location of responsive protons within the structure by medium resolution methods (Rosa & Richards, 1979).

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