Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 10596–10600, December 1991
Biochemistry

Nucleosome positioning is determined by the $(H3\text{-}H4)_2$ tetramer

(histone/chromatin/5S rRNA gene)

FENG DONG* AND K. E. VAN HOLDE†

Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331–6503

Contributed by K. E. van Holde, August 28, 1991

ABSTRACT **It is demonstrated that the histone $(H3\text{-}H4)_2$ tetramer can find specific positions on DNA, even in the absence of other histones. Purified histone $(H3\text{-}H4)_2$ tetramers were reconstituted onto 208-base-pair (bp) DNA molecules containing a nucleosome-positioning sequence by using salt-gradient dialysis. The stoichiometry of histone tetramer to DNA was shown to be 1:1. Digestion with micrococcal nuclease led to formation of protected DNA fragments of ≈73 bp. Cleavage of the 73-bp DNA with restriction enzymes produced a small set of defined bands, demonstrating positioning of the $(H3\text{-}H4)_2$ tetramer on DNA. Analysis of the restriction digests shows that the 73-bp DNA corresponds mainly to two fragments, one lying on either side of the pseudo-dyad axis of the major position adopted by complete histone octamers on this DNA. This result means that a single $(H3\text{-}H4)_2$ histone tetramer can fold ≈146 bp of DNA with the same positioning as the complete octamer but that a region near the pseudo-dyad is only weakly protected against micrococcal nuclease attack in the absence of histones H2A and H2B.**

The ability of histones to select particular sites along a DNA strand on which to form nucleosomes (nucleosome "positioning") is not necessarily surprising. Each possible location along any given DNA strand must, in principle, correspond to a particular value of binding free energy, and some positions will necessarily represent minima in this quantity. That some minima are sufficiently deep to define very specific and stable positions has been demonstrated in many laboratories (for a review of earlier studies, see ref. 1; examples of more recent results are given in refs. 2–9). There are two important questions about positioning: (*i*) what DNA features determine preferred positions? and (*ii*) what portions of the histone octamer are essential to permit selection of a preferred position? The former question has been examined by many workers; answers are as yet incomplete (for recent views, see refs. 7–9).

Only scant attention has been paid to the second question. In a recent study (9), we reported that removal of the N-terminal tails from the core histones had no effect whatsoever on the position adopted by nucleosomes reconstituted onto the 5S DNA sequence from the sea urchin *Lytechinus variegatus*. This result has been confirmed in a very recent report (10). Moreover, recent experiments in our laboratory have strongly suggested that the histones H2A and H2B themselves may not be necessary for determination of nucleosome positioning (9, 11). In a study of the mechanism of salt-gradient reconstitution (11), we have evidence that a regular array of twelve $(H3\text{-}H4)_2$ tetramers is formed upon a dodecameric repeat of the *L. variegatus* sequence as salt concentration is lowered to <1 M. Because further decrease in salt concentration leads to the production of a nucleosome array in which certain positions are strongly favored, this result implies that this positioning has been set by the $(H3\text{-}H4)_2$ tetramers. The very recent report by Hayes *et al.* (10) supports this conclusion via footprinting studies. The experiments described here are designed to test whether this inference is, in fact, correct, by using restriction-cleavage methods and analysis of histone stoichiometry.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

EXPERIMENTAL PROCEDURES

Preparation of DNA Templates and Histone $(H3\text{-}H4)_2$ Tetramer. The tandemly repeated DNA template containing 12 repeats of a 208-base-pair (bp) DNA sequence[§] that includes the *L. variegatus* 5S rRNA gene was prepared as described (9). From this, digestion with *Rsa* I produced the monomeric 208-bp repeating unit (9).

Histone $(H3\text{-}H4)_2$ tetramer was prepared from native nucleosome core particles obtained by micrococcal nuclease digestion of chicken erythrocyte chromatin (12). Histones H2A and H2B were removed from these core particles by sedimentation through a sucrose gradient containing 2 M urea/0.34 M NaCl, as described (12), taking advantage of the fact that the nucleosome core particle dissociates into H2A-H2B dimers and a $(H3\text{-}H4)_2$ DNA complex under such conditions (13). After urea and sucrose were removed by dialysis, the NaCl concentration of the $(H3\text{-}H4)_2$·DNA complex was adjusted to 2 M, and the $(H3\text{-}H4)_2$ tetramer was purified from the DNA by hydroxylapatite column chromatography (14).

Reconstitution of Tetramers onto Defined DNA Templates. We shall call particles in which a single $(H3\text{-}H4)_2$ tetramer has been reconstituted onto a 208-bp DNA fragment "tetramer particles." Reconstitution of tetramer particles was done by using step-wise salt-gradient dialysis under the same conditions as described (9) for reconstitutions of complete nucleosomes. A molar ratio of 0.9 histone tetramer per DNA template was used to minimize the possibility that more than one histone tetramer might associate with one DNA template unit. To ensure correct stoichiometry, two methods were used to determine histone tetramer concentration, one based on the OD of purified histones and a second method using comparison of the amount of H3-H4 in the tetramer with that in native nucleosome core particles. The latter method involved electrophoresis of histones from the tetramer sample and from a nucleosome core particle sample of known concentration on the same SDS/polyacrylamide gel. Core particle concentrations were determined from A_{260} absorbance. Several aliquots of each sample were used, and measurement was made on densitometer scans of the gel after the histones were stained with Coomassie brilliant blue G-250. Thus, the molar concentration of $(H3\text{-}H4)_2$ tetramer

*Present address: Institute of Molecular Biology, University of Oregon, Eugene, OR 97403–1229.
†To whom reprint requests should be addressed.

[§]The length of the cloned DNA sequence containing the sea urchin *L. variegatus* 5S rRNA gene was found to be 208 bp, instead of 207 bp, as previously reported, in plasmid p5S207-12 (recent observation by Borries Demeler in this laboratory). Therefore, the designation p5S207-12 previously used should be corrected to p5S208-12.

was accurately determined from that of the nucleosome core particle solution without influence from the H2A-H2B histones. The concentration obtained in this way compares well with that obtained by the OD method (which was done assuming H3-H4 has approximately the same extinction coefficient at 230 nm as the H2A-H2B). The slightly lower concentration found by the OD method (≈8%) is probably due to a small difference in extinction coefficients between H2A-H2B and H3-H4 histones. Therefore, the concentration determined by densitometer calibration was used for determining the amount of $(H3\text{-}H4)_2$ tetramer stock solution required for the reconstitutions. After reconstitution, the tetramer particles were concentrated by using a Centricon-30 microconcentrator (Amicon) to ≈0.5 mg/ml (DNA weight).

Sedimentation Equilibrium. Sedimentation equilibrium analysis of the reconstituted tetramer particles, as well as complete reconstituted nucleosomes, was done in a Beckman model E ultracentrifuge with computer-interfaced scanner. Experiments were conducted in Tris/EDTA buffer (10 mM Tris·HCl/0.25 mM Na_2EDTA, pH 7.8) at 8000 rpm and at ≈15°C. The experiments were continued until molecular mass calculated from successive scans (usually recorded at intervals of several hours) agreed within experimental error. Sedimentation equilibrium data was analyzed with the following equation:

$$\ln\frac{C(r)}{C(o)} = \frac{\omega^2 M(1-\bar{\nu}\rho)(r^2 - r_o^2)}{2RT},$$

where $C(r)$ is the concentration of the macromolecules at a given point r, whereas $C(o)$ is the macromolecule concentration at the meniscus (r_o). Values of $\bar{\nu} = 0.60$ cm^3/g and 0.66 cm^3/g were calculated for the tetramer particles and complete nucleosomes, respectively. Runs were performed at concentrations of ≈0.4–0.5 mg/ml (DNA weight) for both the tetramer particles and the complete nucleosomes. This rather high concentration was chosen because this was the concentration used in the analyses described below.

Determination of Tetramer Positions on DNA Template. Positions of tetramer on the DNA template were determined by using the method of restriction enzyme mapping that we have used previously for determining nucleosome positions (9), with minor modifications. The reconstituted tetramer particles were first digested with micrococcal nuclease to remove unprotected DNA sequences, and then the position of tetramer structures was determined by analyzing the products of restriction enzyme digestion of the protected DNA sequences after these sequences were purified.

Micrococcal nuclease digestion. The reconstituted tetramer particles were digested with micrococcal nuclease in the presence of 1 mM $CaCl_2$ in 10 mM Tris·HCl buffer, pH 7.5. Digestions were done at either 37°C or 20°C. Preliminary digestion experiments at 37°C indicated that these particles are less stable than the complete nucleosomes and become overdigested easily; therefore, the large-scale digestions were done at 20°C after optimal conditions of digestion were determined by a pilot digestion at this temperature. Digestions were done with the tetramer particles (DNA weight) at 0.4–0.5 mg/ml and micrococcal nuclease of either 50 units/ml (37°C) or 200 units/ml (20°C). The 4-fold higher concentration of the enzyme was used at 20°C to compensate for the lower enzymatic activity at this temperature. Under such substrate and enzyme concentrations, rates of digestion at the two temperatures are approximately the same, but more stable intermediate forms were obtained at the lower temperature. The digestions were stopped by mixing the reaction solutions with 0.5 M EDTA, pH 8.0, to a final EDTA concentration of 20 mM.

Restriction enzyme mapping of the DNA protected by the tetramer. After aliquots of the reconstituted particles were digested at 20°C for the optimal time (≈25 min) established by the pilot digestion, histones were removed by digestion with Pronase in the presence of SDS. The DNA fragments were then electrophoresed through 12% polyacrylamide gels, and certain DNA sequences protected by the $(H3\text{-}H4)_2$ tetramer were purified by the method of Maxam and Gilbert (15). The purified sequences were then digested with selected restriction enzymes to determine the locations of these protected sequences on the original DNA template. To ensure complete restriction digestions, aliquots of the tandemly repeated DNA templates containing the *L. variegatus* 5S rRNA gene sequence [the 208–12 oligonucleosome template, prepared from the plasmid p5S208-12 as described (16)] were digested under the same conditions as a control. Restriction digests were electrophoresed on 12% polyacrylamide gels and analyzed using a described method (9).

RESULTS

Characterization of Tetramer Particles. For these studies to be of significance, two facts must be established: (*i*) only H3 and H4 (in equimolar amounts) are present in the particles, and (*ii*) each particle contains only one $(H3\text{-}H4)_2$ tetramer. The latter is especially important, because Camerini-Otero *et al.* (17) have shown that at high protein/DNA ratios particles can be formed that contain two $(H3\text{-}H4)_2$ tetramers (see also refs. 18, 19). Fig. 1*A* demonstrates the protein composition of the tetrameric particles; only H3 and H4 are present, and the staining intensities indicate equimolar quantities of these. The sedimentation equilibrium data of Fig. 1*B* indicate approximate homogeneity of the sample; the molecular mass value obtained (1.92×10^5 g/mol) agrees well with that expected for a particle containing one histone tetramer and 208 bp of DNA (1.90×10^5 g/mol). In a parallel control experiment, an entirely reconstituted mononucleosome had a value of 2.35×10^5 g/mol, again in good agreement with the expected value, 2.45×10^5 g/mol.

Micrococcal Nuclease Digestion of the Tetramer Particles. As compared with the complete nucleosome core particles containing all eight histone molecules, the tetramer particles are much more sensitive to nuclease digestion. Fig. 2 shows that micrococcal nuclease digests the tetramer particles rapidly at 37°C, generating a ladder of DNA fragments mostly ranging from 70 to 150 bp in length. These results are similar to those obtained by Camerini-Otero *et al.* (17, 21) and by Read *et al.* (22), using particles containing $(H3\text{-}H4)_2$ tetramers on random nucleosomal DNA sequences. Digestion by micrococcal nuclease at a lower temperature and a higher enzyme concentration significantly improves the digestion patterns because a lower temperature stabilizes the tetramer structures (see Fig. 2). However, even at 20°C, these particles still appear much less stable than complete nucleosomes toward micrococcal nuclease digestion. At both temperatures, particles containing protected DNA sequences ≈70 bp in length were obtained as a limiting product; the protection appeared stronger at 20°C than at 37°C. The band is not entirely homogeneous but seems to consist mainly of material 73 ± 2 bp in size. Others have also found that ≈70-bp DNA sequences are strongly protected by the $(H3\text{-}H4)_2$ tetramer toward either DNase I or micrococcal nuclease digestions (17, 21, 22).

The pattern of DNA fragments shown on the 12% polyacrylamide gel electrophoresis differs distinctively from that produced by micrococcal nuclease digestion of free DNA containing the same sequences (data not shown). Naked DNA sequences appear much more accessible to digestion, and cleavages occur much faster and more randomly. In contrast, the production of strong, sharp bands in digests of

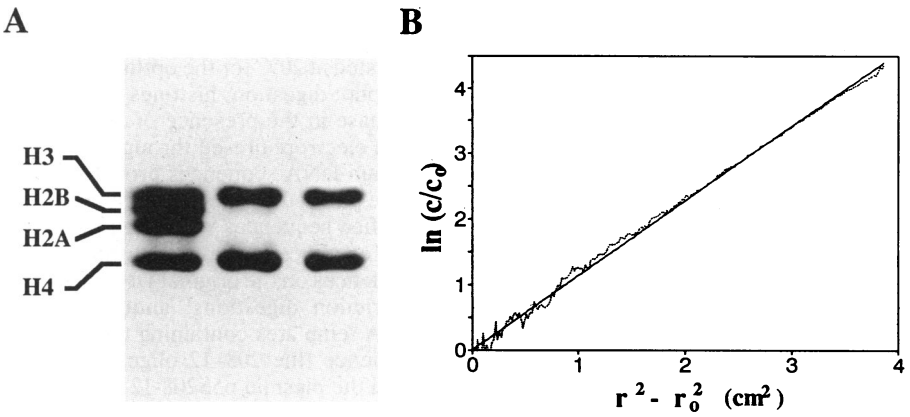

FIG. 1. (*A*) Characterization of proteins used. Gel electrophoresis of histones was done in SDS/15% polyacrylamide slab gels in a discontinuous buffer system by the Laemmli method (20). Lanes: left, core histones in chicken erythrocyte nucleosomes used as standards; center, purified $(H3\text{-}H4)_2$ histone tetramer used for reconstitutions; right, histones in the reconstituted tetramer particles. (*B*) Sedimentation equilibrium analysis of the tetramer particles used. Data are from an average of four scans taken ≈80 hr after start of the run. ···, Interpolated experimental data points; —, linear regression of experimental data.

the tetramer particles indicates existence of either hypersensitive sites or pauses in digestion. This result is especially true in the digestions at lower temperature (20°C). The persistence of a band near 70 bp has been postulated by some researchers to result from protection of an "inner region" of the nucleosomal DNA by the $(H3\text{-}H4)_2$ tetramer (see, for example, refs. 17 and 22). However, an alternative possibility is that this band arises from strongly preferred cleavage near the pseudo-dyad axis in these particles; an idea like this was proposed as early as 1976 (21). We shall discuss this point later.

Determination of Positioning of $(H3\text{-}H4)_2$ Tetramers on DNA. To determine whether or not the $(H3\text{-}H4)_2$ tetramers occupy a limited set of positions on the DNA and to locate such positions, we have subjected these purified fragments to analysis by the method of restriction enzyme mapping (9). In this method, protected DNA fragments are purified from the electrophoresis gels and then digested by a restriction enzyme known to cut once within the sequence. In this case, each position occupied by the tetramer structure should yield two (and only two) DNA fragments, summing to the same total length as that of the protected fragment used in restriction enzyme digestion. If multiple positions are occupied, multiple pairs of bands will appear. If positioning of the $(H3\text{-}H4)_2$ tetramer on the DNA were random, only a smear would result upon digestion. Thus, the results of Fig. 3 clearly indicate that the $(H3\text{-}H4)_2$ tetrameric subnucleosomal particles are positioned on the DNA template. Furthermore, as in the situation with complete nucleosomes, multiple positions were observed.

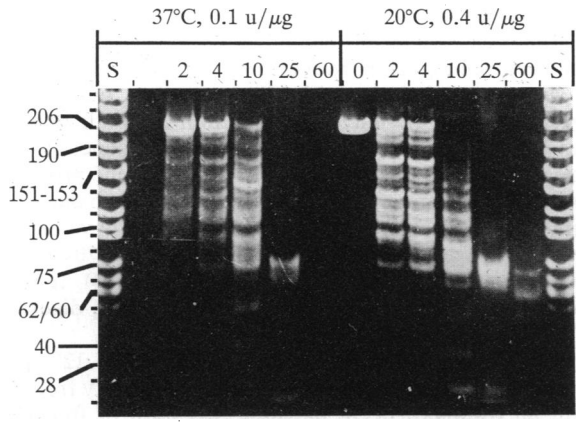

FIG. 2. Gel electrophoresis of micrococcal nuclease digestion of tetramer particles. Digestion condition [temperature, enzyme/substrate ratio of units (u) per μg] and time (in min) are indicated. Lanes labeled S contain DNA standards.

Location of the 73-bp-Protected Fragments. The data shown in Fig. 3 were obtained by using the 73-bp fragment that appears as the major pause in tetramer particle digestion by micrococcal nuclease. When we compare the results of digestion by different restriction enzymes, a remarkable pattern is observed. According to the map at top of Fig. 3 (which shows the major position of complete nucleosomes as a shaded bar), *Alu* I would cleave the 73-bp DNA nearly in half were it a centrally located fragment. In fact, *Alu* I produces very little cleavage—only, at most, a slight trimming at the ends (compare with the "uncut" lane). Control experiments showed that the *Alu* I (as well as all other restriction enzymes used) was fully active under the conditions used (data not shown). On the other hand, the enzymes *Xmn* I and *Msp* I, which should not have cut a centrally located 73-bp sequence, produce well-defined internal cuts in these fragments. The observation that both *Msp* I and *Xmn* I, which have cutting sites >100 bp apart, cleave the 73-bp DNA in this way can be explained only if the 73-bp fragment really consists of two fragments, one from either side of the pseudo-dyad axis of the major position. This interpretation is supported by the fact that although neither *Msp* I nor *Xmn* I will cleave all of the 73-bp DNA, a mixture of the two will do so (Fig. 3, lane 3).

Because about half of the 73-bp DNA is left uncleaved by either *Msp* I or *Xmn* I alone, we conclude that these two fragments are present in pairs of about equal amount. The simplest interpretation is that these represent sequences adjacent to each other on the template, adding up to the length of a complete nucleosome (for example, the sequence 1–73 and the sequence 74–146). Therefore, it seems that the 73 ± 2-bp DNA sequences are a consequence of the protection of a nucleosomal-length DNA sequence (146 bp) combined with preferential cleavages on DNA at or near the pseudo-dyad position of the tetramer particle [see Camerini-Otero *et al.* (21)].

It is difficult to derive precise information concerning localization of this putative 146-bp-protected region on the 208-bp sequence. Although the fragments obtained upon cleavage of the 73-bp fragments can, in general, be assigned to the same major (and some minor) positions observed for the octamer positions, in support of the above model, the pattern is more complex than that we have seen with positioned nucleosomes (9). This complexity arises, in part, from heterogeneity in the 73-bp fragments themselves, which

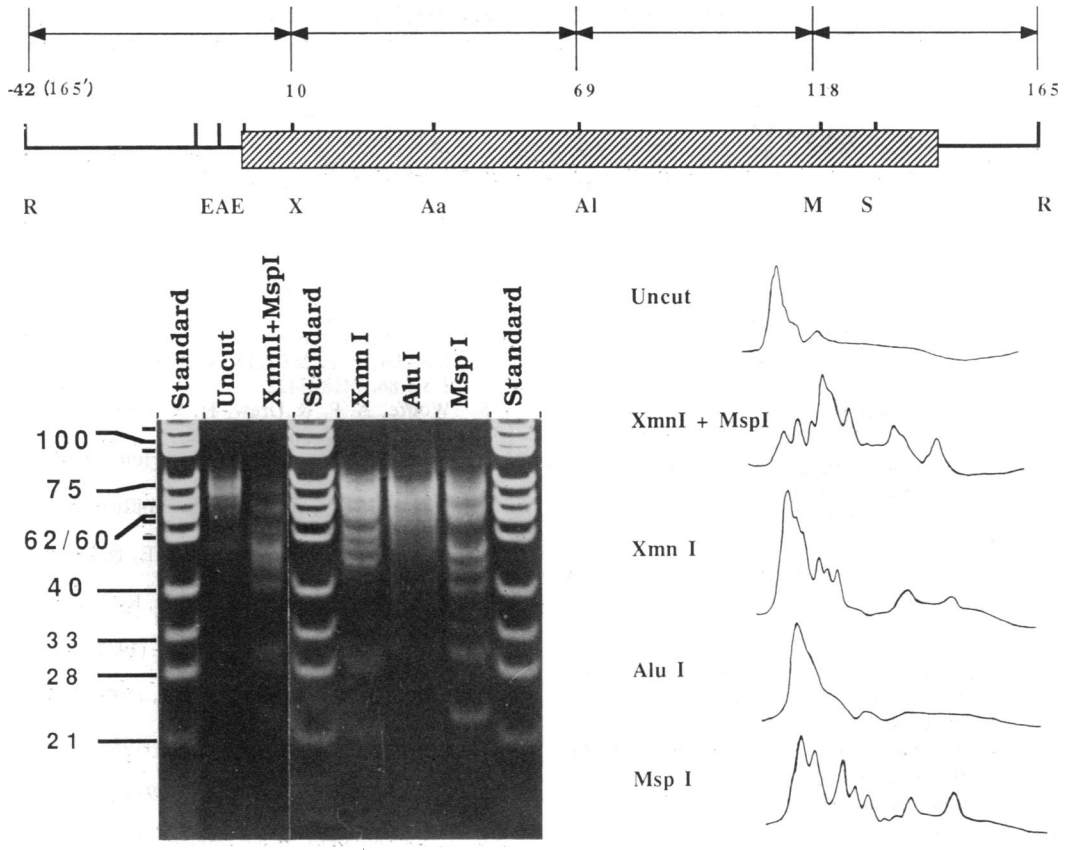

FIG. 3. (*Upper*) Restriction map of the *Rsa* I monomeric DNA template. Shaded band indicates the major position of a histone octamer on this fragment, as determined previously (9). R, *Rsa* I; E, *Eco*RI; A, *Ava* I; Aa, *Aat* II; Al, *Alu* I; M, *Msp* I; and S, *Sau*3AI. (*Lower Left*) Gel electrophoresis of the 73 ± 2-bp-protected DNA (labeled as uncut) and the products of restriction digestion (enzyme for digestion is indicated above each lane). (*Lower Right*) Scans of gel at *Left*.

results from either alternative cleavage positions near the pseudo-dyad or less well-defined end-protection.

We have also purified DNA fragments that are ≈100 bp in length and examined these fragments by restriction enzyme digestion (data not shown). Results suggest that these DNA fragments correspond to sequences of the positioned tetrameric structures that had been cleaved by the nuclease at positions ≈30 bp to either side of the pseudo-dyad, but in which the pseudo-dyad cleavage had not yet occurred. Such results are consistent with the above model. These results also suggest that, as compared with complete nucleosome structures, the DNA in the tetrameric particles is more accessible not only at the pseudo-dyad but at other regions as well. The predominance of the 73-bp DNA fragments in the micrococcal nuclease digestion at late times indicates, however, that the position at the pseudo-dyad is more sensitive than are other regions within the 146-bp-protected sequence.

DISCUSSION

The primary result of this study is the unequivocal demonstration that the $(H3\text{-}H4)_2$ tetramer *alone* can establish positioning on DNA during salt-gradient reconstitution. This conclusion agrees with the inference derived from our earlier reconstitution experiments (11) and from the recent experiments of Hayes *et al.* (10). Together with the demonstrations that histone tails are unimportant in fixing nucleosome position (9, 10), this result means that a surprisingly small number of interactions, contained in the globular portions of two H3 and two H4 histone molecules, are sufficient to determine nucleosome positioning.

This result has major implications for processes in which H2A and H2B are thought lost or exchanged *in vivo* (23, 24); were the tetramer sufficient to retain nucleosome positioning, such processes would not disturb the existing chromatin arrangement.

A second major implication is that the DNA region protected by the histone octamer consists of two portions of ≈73 bp each on either side of the pseudo-dyad axis. This result is surprising, for earlier studies with micrococcal nuclease digestion of end-labeled DNA seemed to show that the protection was limited to a centrally located 70-bp region, as indicated in Fig. 4*B* (see, for example, ref. 22). However, such a position is completely inconsistent with the ability of *Xmn* I and *Msp* I to cut the 73-bp fragments. Instead, our data are consistent with Fig. 4*A*, which suggests that the removal of H2A and H2B exposes a site or sites close to the pseudo-dyad axis to micrococcal nuclease attack. In this model, the entire 146 bp of DNA corresponding to that in the core particle is wrapped about the tetramer, a conclusion in agreement with several other studies (17–19, 21, 22).

An alternative explanation might be advanced: If *two* $(H3\text{-}H4)_2$ tetramers were to bind to the DNA as shown in Fig. 4*C* or some other comparable conformations, they might thus each protect a region of ≈73 bp. Aside from the question why the tetramer would choose a different position than it does in nucleating the deposition of an octamer, this proposal is nullified by the sedimentation analysis (Fig. 1*B*). The sample appears homogeneous, with a molecular mass very nearly that predicted from binding of a single tetramer on each DNA molecule; we have no significant population of particles containing two tetramers under the conditions used.

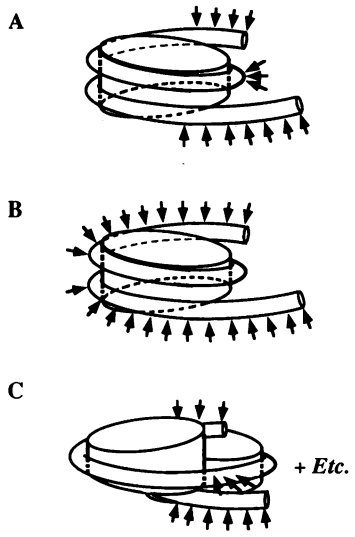

FIG. 4. Three models to explain protection of ≈70-bp DNA fragments by $(H3\text{-}H4)_2$ tetramers. Arrows indicate postulated regions of easier accessibility by micrococcal nuclease.

Another conceivable way of tetramer formation that could yield the positioning results is the existence of two major tetramer populations, present in equal amounts, each covering 73 bp (on separate molecules) to either side of the expected position of the pseudo-dyad of the nucleosome. Although such a model is not logically impossible, it is very unlikely because such manner of tetramer formation requires the tetramers not only to protect DNA sequences exactly half the length of a nucleosome (73 bp) but also to recognize the 73-bp DNA fragments to either side of the full nucleosome position sequence and to bind the two fragments with equal probability. This model also brings about the same question (as for the model in Fig. 4*C*) of the roles of tetramer in nucleating nucleosomes (see above). Finally, such a model, with necessarily equal affinities of the two half-sites for the tetramer, should result in a significant population of two-tetramer structures as the saturation of either site is approached.

We thank Dr. B. Demeler for sequencing the 208-bp DNA fragment. This research was supported by U.S. Public Health Service Grants GM 22916 and PO1 ES04766. K.E.v.H. is the recipient of an American Cancer Society Research Professorship.

1. van Holde, K. E. (1988) *Chromatin* (Springer, New York), pp. 289–317.
2. FitzGerald, P. C. & Simpson, R. T. (1985) *J. Biol. Chem.* **260,** 15318–15324.
3. Linxweiler, W. & Hörz, W. (1985) *Cell* **42,** 281–290.
4. Drew, H. R. & Travers, A. A. (1985) *J. Mol. Biol.* **186,** 773–790.
5. Ramsay, N. (1986) *J. Mol. Biol.* **189,** 179–188.
6. Satchwell, S. C., Drew, H. R. & Travers, A. A. (1986) *J. Mol. Biol.* **191,** 659–675.
7. Shrader, T. E. & Crothers, D. M. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 7418–7422.
8. Wolffe, A. P. & Drew, H. R. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 9817–9821.
9. Dong, F., Hansen, J. C. & van Holde, K. E. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 5724–5728.
10. Hayes, J. J., Clark, D. J. & Wolffe, A. P. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 6829–6833.
11. Hansen, J. C., van Holde, K. E. & Lohr, D. (1991) *J. Biol. Chem.* **266,** 4276–4282.
12. Ausió, J., Dong, F. & van Holde, K. E. (1989) *J. Mol. Biol.* **206,** 451–463.
13. Sibbet, G. J. & Carpenter, B. G. (1983) *Biochim. Biophys. Acta* **740,** 331–338.
14. Simon, R. H. & Felsenfeld, G. (1979) *Nucleic Acids Res.* **6,** 689–696.
15. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 499–560.
16. Hansen, J. C. & Rickett, H. (1989) *Anal. Biochem.* **179,** 167–170.
17. Camerini-Otero, R. D., Sollner-Webb, B., Simon, R. H., Williamson, P., Zasloff, M. & Felsenfeld, G. (1977) *Cold Spring Harbor Symp. Quant. Biol.* **42,** 57–76.
18. Klevan, L., Dattagupta, N., Hogan, M. & Crothers, D. M. (1978) *Biochemistry* **17,** 4533–4540.
19. Thomas, J. O. & Oudet, P. (1979) *Nucleic Acids Res.* **7,** 611–623.
20. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
21. Camerini-Otero, R. D., Sollner-Webb, B. & Felsenfeld, G. (1976) *Cell* **8,** 333–347.
22. Read, C. M., Baldwin, J. P. & Crane-Robinson, C. (1985) *Biochemistry* **24,** 4435–4450.
23. Baer, B. W. & Rhodes, D. (1983) *Nature (London)* **301,** 482–488.
24. Jackson, V. (1987) *Biochemistry* **26,** 2315–2325.