Neutron and X-ray Scatter Studies of the Histone Octamer and Amino and Carboxyl Domain Trimmed Octamers*

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The structure of the nucleosome has been under intense investigation using neutron crystallography, x-ray crystallography, and neutron solution scattering. However the dimension of the histone octamer inside the nucleosome is still a subject of controversy. The radius of gyration ($R_g$) of the octamer obtained from solution neutron scattering of core particles at 63% $\text{H}_2\text{O}, 37\% \text{D}_2\text{O}$ is 33 Å, and x-ray crystallography study of isolated histone octamer gives a $R_g$ of 32.5 Å, while the reported values using x-ray crystallography of core particles from two individual studies are 29.7 and 30.4 Å, respectively. We report here studies of isolated histone octamer and trypsin-limited digested octamer using both neutron solution scattering and small angle x-ray scattering. The $R_g$ of the octamer obtained is 33 Å, whereas that of the trimmed octamer is 29.8 Å, similar to the structure obtained from the crystals of the core particles. The N-terminal domains of the core histones in the octamer have been shown by high resolution nuclear magnetic resonance (Sehroth, G. P., Yau, P., Imai, B. S., Gatewood, J. M., and Bradbury, E. M. (1990) FEBS Lett. 268, 117-120) to be mobile and "not seen" by x-ray crystallography.

The fundamental repeating subunit of eukaryotic chromosomes, the nucleosome, contains the histone octamer ([H3$\alpha$ H4$\alpha$] (H2A, H2B)), one histone H1, and 195 ± 5 base pairs of DNA for many but not all cells. Nucleosome digestion of nucleosomes revealed two well defined subnucleosomal particles: the chromatosome with the full complement of histones and 168 base pairs of DNA, and the core particle containing only the core histone octamer and 146 base pairs of DNA. The structure of the core particle in solution and in crystals has been studied intensively.

Neutron scatter contrast matching studies have given a low resolution structure of the core particle in solution (1-5). The stringency of the neutron scatter data allows the core particle to be described as a flat disc with a diameter of 110-115 Å and thickness of 55-60 Å. It contains 1.7 ± 0.2 turns of DNA of pitch 30 Å coiled around a protein core composed of the apolar structured central domains of the histones in the octamer. The low resolution neutron scatter structure of the core particle in solution is very similar to the original x-ray crystal structure of the core particle at 7-Å resolution (6), to a more recent proposed structure at 8-Å resolution (7), and to the neutron diffraction structure at 25-Å resolution (8). One major unresolved question concerns the shape of the histone octamer which is different in each of the crystal structures of the core particle and different also in the crystal structure of the isolated octamer at 3.3-Å resolution (9). In previous neutron scatter studies of the core particle, it was suggested that the histone octamer was in the form of a cylinder of diameter 70 Å and height 40 Å (3). This shape, however, has a calculated radius of gyration ($R_g$) of 27.3 Å which is substantially smaller than the neutron scatter-determined $R_g$ of the histone octamer at the DNA contrast-matched position of 33-34 Å (1-5). To account for this difference, it was proposed that the well defined N-terminal domains of the histones, which account for 20-25% of the histone mass, extend out from an octameric complex made up of the globular central domains of the core histones (3, 5). In the 7-Å resolution x-ray crystal structure the observed protein electron density is contained within a shape that can be described as a cylinder (diameter 70 Å and height 56 Å). The calculated $R_g$ is 29.7 Å, substantially smaller than the observed $R_g$ of 33 Å. Not all of the expected histone electron density is accounted for in the crystal structure because the "fractional volume of the crystal occupied by the core particle is 0.35 as compared with a calculated value of 0.40" (6). This difference has been attributed to local disorder in the N-terminal domains of the core histones. In the 8-Å x-ray crystal structure it is claimed that 93% of the expected histone density is observed and that the calculated $R_g$ of the histone shape is 30.4 Å (7), again smaller than the neutron scatter-determined value of 33 Å. In this crystal structure, fingers of histone electron density extend out between the DNA gyres and also make intercore particle DNA contacts. Similar extensions of histone density between the DNA gyres have also been observed in the neutron diffraction crystal structure (8).

The crystal structure of the isolated histone octamer has been determined to 3.3-Å resolution (9), and its shape differs
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from the shapes of the octamer in the core particle crystal structures. The crystal shape of the isolated octamer has been described as a prolate ellipsoid with a long axis of 110 Å and short axis of 65–70 Å. Its calculated $R_i$ is 32.5 Å, similar to the observed neutron scatter $R_o$ of the octamer in the core particle. The difference in shape between the structure of the free octamer (9) and the octamer in the core particle crystal structures has been discussed (10–12). One suggestion is that the octamer shape is dependent on ionic conditions, and a preliminary neutron scatter report has been presented in support of this view (12). Another proposal (15) is that the differences in octamer shape outlined above are due to the behaviors of the basic N-terminal domains of the core histones.

There is much evidence to support the view that the well defined basic N-terminal domains of the core histones have distinct structural and functional roles. All of the reversible acetylations of the core histones in the N-terminal domains are associated with all aspects of DNA processing: transcription, replication, and spermiogenesis (see Ref. 13 for review). Nuclear magnetic resonance spectroscopy studies of histone dimers (14) and histone tetramers (15) show that the N-terminal domains are flexible and mobile on the NMR time scale. NMR studies of core particles have shown that the N-terminal domains of H2A and H2B are mobile under low salt conditions; the N-terminal domains of H3 and H4 are more weakly bound than the globular domains and can be released between 0.3 and 0.6 M NaCl (16). The initial x-ray crystal structure of the core particle at 20 Å resolution was of core particles that had been proteolyzed inadvertently (17) and, most probably, had lost their N-terminal domains. It has been shown further that it is possible to trypsin digest the histone N- and C-terminal regions from core particles with little effect on core particle structure (18, 19) and to re-form core particle structures with histone tetramers trimmed of their N-terminal domains (20, 32). Controlled proteolytic digestion studies have shown that the N-terminal domains of H2A (21), H2B (22), and H3 and H4 (23) can be removed selectively from histone complexes and core particles leaving the central globular domains intact. Recent NMR studies comparing control and trypsin-digested histone octamers have shown that all the mobile resonances in the histone octamer are in the trypsin-sensitive domains (33). Although the NMR time scale is orders of magnitude shorter than the x-ray and neutron time scale, the observation of sharp NMR resonance peaks from the N-terminal domains of the histone octamer of $M_i$, 120,000 in the core particle of $M_i$, 220,000 shows unambiguously that these domains are not immobilized by specific bindings in these complexes, but are mobile in the NMR time scale, i.e. rapidly rotating about single bonds. On the neutron or x-ray scatter time scale these techniques would “see” a time average of all possible conformations of their N-terminal domains around the core complex of the spacial domains of the histones. Thus neutron and x-ray scatter will determine directly the effects of removal of these mobile N- and C-terminal domains on the histone octamer radius of gyration. It should be noted also that the core particle contains 146 base pairs of DNA compared with 195 ± 5 base pairs in most nucleosomes. Thus regions of the core histones that interact with the flanking DNA segments in the nucleosome would in the core particle have lost their native DNA binding sites and become free or bind nonspecifically to other core particle DNA or to DNA sites provided by the crystal packing of the core particles. It is reasonable to expect that such regions could include the N-terminal domains of the core histones.

Here we report x-ray and neutron scatter studies of the shapes of histone octamers from different sources and an x-ray scatter study of histone octamers trimmed of their N-terminal domains and C-terminal tails. The trimmed octamer gives an $R_i$ value that is in full accord with the calculated $R_i$ values for the observed histone octamer electron density in the core particle crystal structures.

MATERIALS AND METHODS

Isolation of Nuclei

Chicken erythrocyte nuclei were prepared from packed red blood cells (Pel-Freez Biologicals, Rogers, AR and New American Poultry, Sacramento, CA) by the method of Murray et al. (24). Culture of HeLa cells and isolation of HeLa cell nuclei were by the method of Yagci et al. (25). All nuclei were stored frozen at −80 °C until use.

Calf Thymus Histone Octamer Isolation (Method 1)

Chromatin and core histone octamers were isolated from calf thymus nuclei essentially as described earlier (26, 27). The octamers were stored as 60% ammonium sulfate precipitates. Prior to use, the precipitates were resuspended in the desired buffer and dialyzed against the same buffer. The samples were clarified by ultracentrifugation at 100,000 × g for 30 min.

Chicken Erythrocyte and HeLa Cell Trypsin-trimmed and Untrimmed Histone Octamer Isolation (Method 2)

Isolation of H1/H5-depleted Soluble Chromatin—Chicken erythrocyte nuclei (4 mg/ml in DNA) were digested at 37 °C for 25 min with 25 units of micrococcal nuclease/mg of DNA in 10 mM Tris-HCl, pH 7.4, 1 mM CaCl₂, 1.5 mM MgCl₂, 0.25 M sucrose, and 0.1 mM phenylmethylsulfonyl fluoride. HeLa nuclei were digested for 15 min under the same conditions. The digestion was terminated with the addition of EDTA to 2 mM. The nuclei were resuspended in lysis buffer (10 mM Tris-HCl, pH 7.4, 0.5 mM EDTA, and 0.1 mM phenylmethylsulfonyl fluoride) and dialyzed against the same buffer overnight at 4 °C to obtain solubilized chromatin.

The solubilized chromatin was clarified by centrifugation at 6000 × g for 10 min and was applied to a hydroxyapatite column (Calbiocem, fast flow) equilibrated with 0.12 M sodium phosphate. Histone H1/H5-depleted chromatin was eluted from the column with a linear gradient of 0.12–0.45 M sodium phosphate (equimolar mono- and dibasic sodium phosphate) using a Pharmacia FPLC system. The H1/H5-depleted chromatin peak was pooled, dialyzed against 10 mM Tris-HCl, pH 7.4, 0.1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, and concentrated to 2 mg/ml (DNA) with a 50K MWCO ultrafiltration membrane (Pall) in an Amicon TCF-10 apparatus.

Trypsinized Core Particles—The H1/H5-depleted chromatin was made 1 mM in CaCl₂ and redigested with 40 units of micrococcal nuclease/mg of DNA at 37 °C for 25 min. The digestion was terminated with the addition of EDTA to 2 mM; the chromatin was concentrated by ultrafiltration prior to loading on 5–30% sucrose gradients. The gradients were spun in a Beckman SW 28 rotor at 26,000 rpm for 21 h at 4 °C. The fractions containing core particles were pooled and dialyzed against the same buffer as above. The core particles (2 mg/ml in DNA) were digested with 1 mg/ml trypsin (Sigma, L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated) for 6.5 h at 4 °C. The digestion was terminated with the addition of 1-chloro-3-tosylamido-7-amino-2-heptanone (Sigma) to 20 µg/ml. The samples were concentrated and repurified on sucrose gradients as described above.

Octamers and Trypsin-trimmed Octamers—Control or trypsinized core particles were loaded on a hydroxyapatite column equilibrated with 50 mM sodium phosphate, pH 7.7, 0.1 mM phenylmethylsulfonyl fluoride and eluted with 2 mM NaCl in the same buffer. The isolated trimmed or control untrimmed histone octamers were concentrated by ultrafiltration and dialyzed against 2 M NaCl in preparation for neutron and x-ray scattering analysis. Fig. 1 shows the polyacrylamide gel electrophoretic patterns of the trimmed and untrimmed histone octamers. These patterns are identical to those published previously of fully characterized trimmed histone octamers (21-23, 32).
Structure of Trypsinized Histone Octamer

Sample Preparation for Neutron Scattering

One day prior to the neutron scattering experiments, the samples were dialyzed extensively against either 100% D2O or 100% H2O. Samples with intermediate D2O concentrations of 63, 40, and 25% were made by mixing appropriate ratios of the 100% D2O and 100% H2O samples. Histone octamer solution concentrations were measured by their absorbance at 277 nm, as described elsewhere (27).

X-ray Scattering

The x-ray scatter studies were carried out at the synchrotron radiation source (DESY) in Hamburg. A wavelength of 0.15 nm and three camera lengths of 4, 1.5, and 1 m gave a momentum transfer (Q) range of 0.125-2.9 nm⁻¹. A quadrant detector was used to enhance the high angle data. The Q values were calibrated using known diffraction peaks from rat tail collagen and absolute values of scattering intensity obtained by normalizing to the scattering from lysozyme. Samples were studied at room temperature. Guinier plots from the 4-m data were linear over a wide range of Q, and radii of gyration were determined from linear fits over the Q range of 0.26-0.55 nm⁻¹.

Neutron Scattering

The neutron experiments were performed on the D11 and D17 instruments at the Institut Laue Langevin in Grenoble, France (34). The neutron wavelength was 1.0 nm on D17, and two camera lengths, 3.41 and 0.8 m, gave a range of accessible Q values from 0.09 to 2.6 nm⁻¹. Linear fits to Guinier plots were made over the range of 0.22-0.35 nm⁻¹. Samples were contained in quartz cells 1 mm thick (Hellma) and maintained at 8°C. The momentum transfer Q for each detector element was calculated from the geometry, and after correction for the scattering from the cell, intensities were normalized by the incoherent water (D2O) scatter so that absolute cross-sections could be determined (35). I(Q) versus Q scattering curves for the whole Q range were obtained by concatenating the spectra from the two camera settings and subtracting the incoherent background.

RESULTS

Neutron and X-ray Scatter Study of the Histone Octamers—
The neutron scatter curve of the isolated chicken erythrocyte histone octamer in 2 M NaCl and 63% D2O, 37% H2O solution (Fig. 2) shows a sharp minimum at an equivalent Bragg spacing of 4.6 nm and a maximum at an equivalent spacing of about 3.6 nm. This scatter curve and the equivalent Bragg spacings are identical to those observed in the neutron scatter curve of the core particle at the DNA contrast-matched position (3-5). The similarity of these scatter curves argues against the possibility that the residual internal structure of the DNA at the contrast-matched position is responsible for the higher value of the octamer Rv in the core particle when compared with the calculated Rv values for the histone octamer in the crystal structure (6, 7).

Fig. 2. Neutron scatter curve of the isolated histone octamer from chicken erythrocytes in 2 M NaCl and 63% D2O. The ordinate is in units of dσ/dΩ · M/4π Na d where dσ/dΩ is the differential scattering cross-section per particle, M is the molecular weight, Na is Avogadro’s number, and d is the cell thickness of 1 mm. Note that the concentration of this sample was so high that interparticle effects are apparent at very low angle.

Fig. 3. Guinier plots of the isolated histone octamer from calf thymus in 2 M NaCl and various contrasts. The ordinate is as in Fig. 2.
octamer in 2 M NaCl are consistent with the neutron measurements.

The intersect of the Guinier plots on the ordinates at zero 
Q varies as the square of the contrast (28, 29). This plot is 
given in Fig. 4 for the calf thymus octamer in 2.0 M NaCl. 
The octamer is contrast-matched at 41% H2O. From the 
slope of the straight line plot and the known molecular mass 
of 109,000 kDa, the "dry" volume of the octamer of 99 nm3 is 
obtained. The dry volume of octamer excludes the volume 
occupied by bound water and exchangeable protons (30).
Allowing for half the volume of a water molecule per ex-
changeable proton, the volume of the dehydrated octamer is 
129 nm3, which agrees very well with a volume of 133 nm3 
based on a partial specific volume of 0.73 cm3/g.

Pair Distance Distribution Functions—A good representa-
tion of the histone octamer particle in solution is given by the 
pair distance distribution function P(R). This function is the 
spherical average of the distribution of all possible chord 
lengths that can be drawn within the envelope of the shape 
of the particle. The P(R) function is obtained by taking the 
sine Fourier transform of the scatter curves of histones octamers in high concentration in 37% 
H2O, 2 M NaCl (Fig. 2) is given in Fig. 5. The P(R) functions 
have been normalized by I(0), the scattering intensity at Q = 0, 
and therefore have an area of unity. The normalization 
procedure is advantageous because the area under the P(R) 
curve is I(0) and is proportional to the molecular weight of 
the octamer. Three octamer samples in 2 M NaCl solutions 
gave identical P(R) curves showing chord lengths up to 
105 Å is thus the maximum dimension of the octamer in 2 M 
NaCl.

X-ray Scatter Studies of Histone Octamers and "Trimmed"

Table I
| Source of octamer | $^3$H2O | R, Å |
|------------------|--------|-----|
| Calf thymus      | 0      | 32.5|
| Chicken erythrocyte | 0   | 33.4|
| Calf thymus      | 63     | 32.0|
| Chicken erythrocyte | 63  | 31.7|
| HeLa cells       | 63     | 32.4|
| Calf thymus      | 100    | 31.5|
| Chicken erythrocyte | 100 | 31.3|

Table II
| Histone                              | Full length | Trypsin protected | Residues lost |
|--------------------------------------|-------------|------------------|---------------|
| Calf thymus H2A                      | 129         | 12-118           | 22            |
| Calf thymus H2B                      | 125         | 24-125           | 23            |
| Calf thymus H3                       | 135         | 27-123           | 32            |
| Calf thymus H4                       | 102         | 18-102           | 17            |

Octamers—The proposal that the behaviors of the basic N-
terminal domains of the core histones are responsible for the 
differences between the shapes of the histone octamer in the 
core particle in solution (1—5), in the core particle crystal 
structure (6—8), and in the octamer crystal (9) was tested by 
comparing the x-ray scatter R, of the histone octamer with 
that of the N-terminal domain trimmed octamer both in 2.0 
M NaCl. Controlled proteolysis of histone complexes results 
in the specific cleavage of the basic N-terminal domains of 
the core histones (21—23, 27). Fig. 1 shows the sodium dodecyl 
sulfate-polyacrylamide gel analysis of the intact octamer (lane 
1) and the trypsin-limited digested octamer (lane 2). As can 
be seen after limited digestion, there are four major bands 
that migrate faster than the intact histone bands. These 
correspond to the N-terminal domain trimmed histones that 
have been characterized fully as shown in Table II (21—23). 
The trimmed histone bands are of equal intensity showing that 
the histone octamer stoichiometry is maintained. Of a 
total number of residues of 491 for the intact octamer, 95 are 
removed by trypsin digestion, i.e. 20%.

The Guinier plots for the intact histone octamer in 0.8, 1.2, 
and 2.0 M NaCl at pH 7.5 for a range of concentrations are 
given in Fig. 6. The integrity of the octamer is maintained 
from 2.0 M down to 0.8 M NaCl, and the R, value is 33.5 ± 0.3 
Å. This is slightly larger than the neutron scatter R, of the 
octamer in 2.0 M NaCl of 32.1 ± 0.4 Å (Table I). This 
difference is probably due to the high proportion of the basic 
lysines and arginines located on the surface of the octamer 
and in the N-terminal domains of the core histones. Lysines 
and arginines have a large number of exchangeable protons 
(4 in lysine and 6 in arginine compared to 1 or 2 in other 
residues) that are replaced by deuterium in proportion to the 
$^3$H2O/$^4$H2O ratio of the aqueous solvent and hence have lower 
contrast to the solvent in neutron scatter. No such contrast 
variation across the particle occurs in x-ray scatter. Assuming 
that all of the arginines and lysines are on the surface of the
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FIG. 6. Guinier plots obtained by x-ray scattering from the isolated histone octamer in NaCl at various concentrations and ionic strengths at pH 7.5. There appears to be no systematic variation in either slope (radius of gyration) or intercept (molecular weight) over the range of parameters studied. Note that the abscissa is in terms of $S (= Q/2 \pi)$ here.

octamer, the neutron $R_g$ would be expected to be 4% smaller than the x-ray $R_g$ of the octamer as is observed.

The x-ray scatter Guinier plots of the histone octamer and trimmed octamer in 2 M NaCl are given in Fig. 7. The trimmed octamer has an $R_g$ value of 29.8 Å compared with 33.5 Å for the intact octamer. From the $I(0)$ value the molecular mass of the trimmed octamer was 73 kDa, about 30% less than the molecular mass of the intact octamer of 109 kDa. The $R_g$ value of 29.8 Å for the trimmed octamer is now the same as that calculated for the $R_g$ of the protein electron density from the core particle crystal structure of 29.7 Å (6). This lends support to the hypothesis that the N-terminal domains are disordered and not seen in x-ray diffraction in the core particle crystal structure.

DISCUSSION

The major differences between the small angle neutron scattering low resolution structure of the core particle in solution (3-5) and the x-ray crystal structure (6) are in the shape of the histone octamer. The small angle neutron scattering determination of the $R_g$ of the octamer in the core particle, at the DNA contrast-matched position, of 33–34 Å (3–5) is substantially larger than the values calculated for the octamer $R_g$ from the electron density of the crystal structure (6) of 29.7 Å. To account for this difference it has been postulated previously that the basic N-terminal domains of all four core histones and the basic C-terminal tails of H2A and H3 extend out to distances larger than the 70-Å diameter $\times$ 57 Å thick electron density shape of the core particle crystal structure (6), probably out to 110–120 Å into the region of the DNA gyres (3, 5). This proposal is based on high resolution NMR studies of core particles that show mobilities of these domains (16) and controlled proteolysis of histone complexes and core particles (18–23, 27) that shows accessibilities to proteolysis of these N- and C-terminal domains. Table I gives the proteolysis-protected histone peptides in histone complexes and core particles. It is well documented that about 20% of the histone octamer mass can be removed precisely from histone complexes and core particles by controlled proteolysis and that the protected core histone peptides lack the N-terminal domains of all four core histones and the C-terminal tails of histones H2A and H3. Thus an explanation for the low calculated octamer $R_g$ values from the core particle crystal structures is that the N- and C-terminal domains are either statically or dynamically disordered and are not seen in the crystal structures. What is seen is the structure of the octameric complex of the apolar globular domains of the core
histones that correspond to the trypsin-resistant peptides given in Table II. A test of these ideas is to determine the effects of trypsin digestion removal of the N- and C-terminal regions (Table II) on the radius of gyration of the histone octamer. The neutron scatter radius of gyration of the octamer in 2 M NaCl is 32.1 Å. The x-ray scatter radius of gyration of the octamer in 2.0 M NaCl is 33.5 Å and, for the reasons given earlier, is expected to be 4% larger than the neutron scatter value. The x-ray \( R_g \) of the trypsin-trimmed octamer is 29.8 Å and accords fully with the calculated \( R_g \) values for the observed histone octamer electron density in the 7-Å core particle structure of 29.7 Å (6) and for the 8-Å structure of 30.4 Å (7). In this latter core particle crystal structure, however, it is claimed that only 7% of the histone electron density remains to be accounted for. This is difficult to reconcile because it is substantially less than the 20% of the core histone N- and C-terminal tails that have been shown repeatedly to be removed by trypsin digestion in chromatin, core particles, and histone complexes (18-23, 27, 32). It is possible that precise but nonphysiological DNA environments are provided in the core particle crystal unit cell for adventitious binding of the basic N- and C-terminal domains of the core histones. A recent laser-induced cross-linking study of histones to DNA in chromatin and core particles provides “direct evidence for (the) interaction of nonstructured tails of core histones with linker DNA" (31). These linker DNA regions have been removed in core particles, and presumably the basic N- and C-terminal domains of the core histones bind nonspecifically to other sites.

From the above discussion it is reasonable to propose that the different shapes reported for the histone octamer in isolation, in core particles in solution, and in crystals are most probably due to the different behaviors of the basic N- and C-terminal domains of the core histones in the different ionic solution and crystal conditions. Fig. 5 gives the pair distance distribution \( P(R) \) function for histone octamers in 2 M NaCl. Calculated \( P(R) \) functions for the different histone octamer shapes reported for the isolated histone octamer crystal structure (9) and the octamer shapes in the 7-Å (6) and 8-Å (7) crystal structures are given in Fig. 8. The calculated \( P(R) \) curves for the histone octamer shapes in the core particle crystal structures are a reasonable fit to the octamer \( P(R) \) curve for the vectors out to 60 Å, but the observed \( P(R) \) curve diverges at larger vectors. An explanation for this divergence is that mobile and disordered N- and C-terminal domains are observed in the neutron scatter curves but not in the crystal structures. Because of their easy accessibility to trypsin these basic N- and C-terminal domains must be on the outside of the octamer complex and in the observed \( P(R) \) curves would be expected to contribute to the longer vectors. An approximation to the shape of the isolated histone octamer crystal structure is given by an ellipsoid 67.5 × 67.5 × 110 Å. This gives a reasonable fit to much of the observed \( P(R) \) curve including the longer vectors. This is to be expected because the maximum chord length is along the axis of the ellipsoid and is 110 Å. As discussed above, trypsin removal of the N- and C-terminal domains from the core histones reduces the octamer \( R_g \) from 33.5 to 29.8 Å, and this lower value accords with the calculated \( R_g \) for the observed electron density of the octamer in the core particle crystal structure. An understanding of the behaviors of these basic flexible histone N- and C-terminal domains appears, therefore, to be essential to the interpretation of the data on histone octamer shapes. Behavior that would provide an explanation for the different shapes
reported for the histone octamer are given in Fig. 9. In the core particle it is to be expected that the basic N-terminal domains released from their previous binding to linker DNA would at low ionic strengths be attracted to the DNA gyres around the core particle (Fig. 9A). These N-terminal regions in the high ionic strengths of the octamer crystal would largely charge neutralized and assume an ordered structure in the isolated octamer crystal structure (Fig. 9B). Because all of the chromatin functions related reversible chemical modifications of the core histones are located in these basic N-terminal domains, it is of considerable importance to identify the sizes of interaction of these well defined domains in nucleosomes and chromatin. Clearly this will require structural studies above the level of the core particle.

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