Hyperacetylation of Core Histones Does Not Cause Unfolding of Nucleosomes

NEUTRON SCATTER DATA ACCORDS WITH DISC SHAPE OF THE NUCLEOSOME*

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Recent studies report that the frictional resistance of partially acetylated core particles increases when the number of acetyl groups/particle exceeds 10 (Bode, J., Gomez-Lira, M. M. & Schröter, H. (1983) Eur. J. Biochem. 130, 437-445). This was attributed to an opening of the core particle though other explanations, e.g. unwinding of the DNA ends were also suggested. Another possible explanation is that release of the core histone N-terminal domains by acetylation increased the frictional resistance of the particle. Neutron scatter studies have been performed on core particles acetylated to different levels up to 2.4 acetates/H4 molecule. Up to this level of acetylation the neutron scatter data show no evidence for unfolding of the core particle. The fundamental scatter functions for the envelope shape and internal structure are identical to those obtained previously for bulk core particles. The structure that gave the best fit to these fundamental scatter functions was a flat disc of diameter 11-11.5 nm and of thickness 5.5-6 nm with 1.7 ± 0.2 turns of DNA coiled with a pitch of 3.0 nm around a core of the histone octamer. The data analysis emphasizes the changes in pair distance distribution functions at relatively low contrasts, particularly when the protein is contrast matched and DNA dominates the scatter. Under these conditions there is no evidence for the unwinding of long DNA ends in the hyperacetylated core particles. The distance distribution functions go to zero between 11.5 and 12 nm which gives the maximum chord length in a particle of dimension, 11 nm × 5.5 nm. The distance distribution function for the histone octamer contains 85% of the vectors within the 7.0-nm diameter of the histone core. 15% of the histone vectors lie between 7.0 and 12.0 nm, and these are attributed to the N-terminal domains of the core histones which extend out from the central histone core. Histone vectors extending beyond 7.0 nm are necessary to account for the measured radius of gyration of the histone core of 3.3 nm. A similar value of 3.2 nm is calculated for the recent ellipsoidal shape of 11.0 × 6.5 × 6.5 nm from the crystal structure of the octamer. However, the nucleosome model based on this structure is globular, roughly 11 nm in diameter, which does not accord with the flat disc shape core particle obtained from detailed neutron scatter data nor with the cross-section radii of gyration of the histone and DNA found previously for extended chromatin in solution.

Neutron scatter studies have given the structure of the nucleosome core particle in solution (1-4). The stringency of the neutron data show that the core particle in solution can be described as a flat disc of diameter 11.0-11.5 nm and thickness 5.5-6.0 nm with 1.7 ± 0.2 turns of DNA of pitch 3.0 nm coiled around a histone octamer core approximately 4.0 x 7.0 nm with N-terminal domains extending out from this core to give the measured radius of gyration (Rg) of the histone octomer of 3.3 nm (4). Within the resolution of the neutron scatter data this solution structure is similar to the recent 0.7-5 (5) and 1.5-6 (6) x-ray crystal structures; however, in the 0.7-nm structure the calculated Rg for the histone core, if it contains all of the histone polypeptide chains, is 2.55 nm and much lower than the observed Rg of 3.3 nm. Neutron diffraction studies of core particle crystals give a similar overall structure to the x-ray structure (7) except that they report regions of protein density extending out from the central histone core into the DNA gyres. Very recently a quite different model has been proposed for the structure of the nucleosome (8). This is based on the x-ray crystal structure determination of the histone octamer at 4.0-A resolution which has an envelope shape of 11.0 × 6.5 × 7.0 nm, larger than the size of the histone core from the neutron scatter (2, 4) and x-ray diffraction (5) studies of the core particle, although in the neutron scatter studies (2, 4) it was necessary to put the N-terminal domains of the core histones between 7.0 and 12.0 nm to account for the measured radius of gyration of the histone core of 3.3 nm and in the neutron diffraction structure histone density extends from a central apolar curve into the region of the DNA coils. In the nucleosomal model based on the histone octamer crystal structure, the DNA is located in grooves on the outside of the octamer to give a nucleosome particle which is approximately globular with a diameter of 11.0 nm. The data obtained in this study and from earlier studies will be compared with this new model.

Neutron scatter techniques are particularly powerful in conformational studies of two component systems such as nucleosomes and chromatin. They have unique application to problems which cannot be addressed easily by structure analysis of single crystals. Such questions relate to the structure of higher order chromatin structures and to changes in nu-

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cleosome and chromatin structures in response to cell functions. Of particular current interest are the structures of active and potentially active chromatin. Extended structures have been described for transcriptionally active chromatin loops (9) and are reported for "active" nucleosome core particles (peak A particles or lexosomes) containing the ribosomal RNA genes of *Physarum polycephalum* (10-12). Various chromatin variables have been associated with the active state of chromatin. These include: (i) core histone acetylation (13); and (ii) the presence of high mobility group proteins 14 and 17 (14-16). The sites of acetylation of core histones are lysines located entirely in their basic N-terminal domains: H2A (lysine 5), H2B (lysines 5, 10, 13, and 19), H3 (lysines 9, 14, 18, and 23), and H4 (lysines 5, 8, 12, and 16) (see Ref. 13). Initially these very basic N-terminal domains were thought to be involved in major interactions with DNA in stabilizing the nucleosome, and acetylation of up to 4 lysines in H2B, H3, and H4 was envisaged to be a mechanism for weakening or suppressing these interactions leading to an unfolding of the nucleosome to a transcriptionally active form of the nucleosome.

An approach to studies of the effects of histone acetylation on nucleosomes and chromatin structure was provided by the finding that sodium butyrate treatment of cells resulted in enhanced levels of histone acetylations (17) through the inhibition of histone deacetylase activity (18). The highly acetylated chromatin was found to have enhanced sensitivity to DNase I (18). The physical properties of isolated acetylated core particles from butyrate-treated HeLa cells, as judged by sedimentation coefficient, DNA thermal melting and circular dichroism did not differ significantly from those of the control core particles (reported in Ref. 18). This led to a re-evaluation of earlier ideas concerning the structural effects of acetylation on the nucleosome and to the suggestion that histone acetylation may affect internucleosomal interactions and be involved in the destabilization of the 34-nm supercoil of nucleosomes (18) as was also suggested by Simpson (19). Studies on fractionated hyperacetylated nucleosomes and oligonucleosomes supported a model in which the basic N-terminal domains of the core histones interact with the DNA at the entry and exit to the nucleosome (20), and studies of the interactions of N-terminal peptides of H4 with DNA gave evidence of an unusual type of interaction which was suppressed by acetylation (21). The location of these core histone N-terminal interactions and their suppression by acetylation suggest mechanisms whereby acetylation could modify chromatin structure.

Other more indirect evidence supports the view that core histone acetylation does not markedly affect core particle structure. Extensive trypsin digestion of histones in core particles is required before the core particles unfold from a compact to an extended form (22). An early low resolution crystal structure determination of the core particle (23) showed that the core histones had been proteolyzed, most probably losing their N-terminal domains. In controlled proteolysis studies of nucleosomes, chromatin, and nuclei, it has been shown that the N-terminal domains were preferentially digested (see Ref. 24). Digestion of these N-terminal domains from H1-depleted chromatin and then adding back H1 gave a chromatin which, unlike the control, was unable to stabilize the 34-nm supercoil of nucleosomes (25). This implicated the core histone N-terminal domains in the stabilization of the 34-nm supercoil either in a direct involvement or by providing the correct binding site for the location of H1.

Recent studies, however, report that the frictional resistance of partially acetylated core particle in nondenaturing gels increased when the number of acetyl groups exceeds 10 (26). This was attributed to an enhanced conformational freedom of the core particles, i.e., an unfolding of the nucleosome which was reported from electron microscope study (27). Other structure changes, e.g., "half-nucleosome nonpairing" or the unwinding of the DNA ends, were also suggested. Another explanation is that the release of the core histone H3 and H4 N-terminal domains by acetylation would probably lead to an increase in the frictional resistance of the particle.

The power of neutron techniques in studies of the structures of core particles (1-4), nucleosomes (1), and chromatin (28) results from the ability to match the scatter length density of either the histones or the DNA by changing the proportions of H2O to D2O in mixed water solutions. Parameters obtained from such nonperturbing experiments include the radius of gyration of the DNA $R_g(DNA)$; the histone $R_g$(histone); the pair distance distribution functions $P(R)$ of the particle and of the DNA and histone components; the basic scatter functions of the particle; and the Stuhmann parameters $\alpha$ and $\beta$ (29, 30). The technique is very sensitive to changes in shape of the core particle and to changes in the spatial arrangement of the DNA and histone components.

Here we report on neutron scatter studies of hyperacetylated nucleosome core particles and control particles.

**EXPERIMENTAL PROCEDURES**

**Preparation and Digestion of Nuclei**—Nuclei were isolated from HeLa S3 cells grown in the presence of sodium butyrate and digested with micrococcal nuclease as previously described (20). The nuclei were digested with micrococcal nuclease (25 units/mg of DNA) at 37°C for 15 min. The nuclei were lysed in lysis buffer (10 mM Tris-HCl, pH 6.85, 10 mM sodium butyrate, 5 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride for 15 min on ice, centrifuged, and the supernatant, which contained the solubilized chromatin, was used in subsequent steps.

**Isolation of Mononucleosomes**—Mononucleosomes were isolated either by sucrose gradient centrifugation or by exclusion chromatography on an A-5m (Bio-Rad) column. 5-20% (w/v) sucrose gradients were run and fractionated as previously described (20). Exclusion chromatography was performed on a 5 x 95-cm A-5m column equilibrated with lysis buffer. The column was run at a flow rate of 40 ml/h, and the mononucleosome-containing fractions were pooled.

**Hydroxyapatite Chromatography**—Chromatin samples, either before or after fractionation into mononucleosomes, were applied to a 35-ml hydroxyapatite (Bio-Rad HTP DNA grade) column equilibrated with 100 mM potassium phosphate, pH 7.0, 10 mM sodium butyrate, 0.1 mM phenylmethylsulfonyl fluoride. The column was washed with 100 ml of the same buffer, and the chromatin was eluted with a 100-350 mM potassium phosphate gradient (100 ml, total volume) at a flow rate of 50 ml/h. The material, eluted at a single broad peak, was split into three roughly equal fractions.

**Sample Concentration**—When necessary, samples were concentrated with an Amicon PM-30 membrane in a stirred pressure cell or dialyzed and concentrated with a Pro-Di-Con vacuum dialysis concentrator (Bio-Molecular Dynamics, Beaverton, OR). The nuclei were then dialyzed against 10 mM Tris-HCl, pH 7.4, 5 mM sodium butyrate in 100 and 0% D2O prior to neutron analysis. Intermediate concentrations of H2O were obtained by mixing appro-
Neutron Scatter Experiments—High and low acetylated nucleosome particles obtained by exclusion chromatography followed by hydroxylapatite chromatography were used in the neutron scatter experiments. Neutron scatter experiments were carried out on instrument D11 at the high flux reactor of the Institut Max von Laue-Paul Langevin, Grenoble (34) using specimen-to-detector distances of 4.08 and 1.08 m. The momentum transfer \( Q = 4 \pi \sin \theta / \lambda \) where \( 2 \theta \) is the full scattering angle and \( \lambda \) is the neutron wavelength was calculated for each value of \( 2 \theta \) so that the scattered intensity \( I(Q) \) versus \( Q \) was obtained over a range of 0.12-2.2 nm\(^{-1}\). Solutions of nucleosome core particles in different \(^1\)H\textsubscript{2}O-\(^2\)H\textsubscript{2}O mixtures were contained in quartz cuvettes of 1.00- or 1.01-mm pathlength. The beam cross-section at the sample position was \( 7 \times 10 \) mm\(^2\). The diffraction probabilities per unit solid angle \( I(Q)/I(Q) \) where \( I_0 \) is the intensity of the incident beam and \( \Delta \Omega \) the solid angle covered by one detector element) were determined by using the calibration of May (35). After matching the two sets of data, the incoherent background level was subtracted and \( \Delta(Q)/\Delta \Omega \). the coherent diffraction cross-section/unit solid angle/ per particle was determined by normalization to the transmission \( (T) \), the cuvette thickness \( (d) \), and the particle density \( n = C\text{DNA}N_A/2M\text{DNA} \) (where \( C\text{DNA} \) is the concentration of DNA in solution as determined by the absorption at 260 nm, \( N_A \) is Avogadro’s number, and \( M\text{DNA} \) is the molecular mass of DNA in the nucleosome particle).

Pair Distance Distribution Function—An indirect Fourier transformation program developed by Glatter (36) was used to transform the neutron scatter data into pair distance distribution function (PDDF) \( P(R) \) in real space. They represent the distribution of vector lengths which can be drawn from all small equal volume elements within this particle in turn to all other similar volume elements, multiplied with the scattering lengths contained in each of the volume elements forming a pair, i.e.,

\[
P(R) = \frac{R}{2\pi} \int_0^\infty Q dQ \sin(\theta(Q)) \frac{\sin(2\pi R/Q)}{2\pi R} dQ
\]

\( P(R) \) becomes zero at a value \( R_{\text{max}} \) which corresponds to the largest vector that can be drawn in the particle, and it reaches a maximum at a value \( R_{\text{min}} \), the most probable length of the vectors which can be drawn in a particle. The monodispersity of the core particle in solution can be demonstrated most easily by this procedure. From scattering data directly it is difficult to detect background effects of aggregates. This indirect transformation also allows for the choice of an appropriate value for the minimum momentum transfer \( Q_{\text{min}} = \Pi/D_{\text{max}} \) which is not influenced by interparticle interference (36). Further, it allows for the evaluation and correction of inconsistencies in the raw data which are due to statistical deviations and due to errors in the subtraction of the background. For example, at high proportions of \(^2\)H\textsubscript{2}O in the \(^1\)H\textsubscript{2}O: \(^2\)H\textsubscript{2}O mixtures the background of neutrons scattered by the buffer deviates appreciably from a constant value at the largest momentum transfers measured because of the coherent cross-section of heavy water (35). The conditions that the distance distribution function has to go to zero at zero distance allows an evaluation of this anisotropic background. Important integral parameters of the scatter curves are the differential cross-section at zero angle and the radius of gyration. These two parameters can be calculated from the PDDFs, the former from the area under the \( P(R) \) curve and the latter is 4\( \pi \) times the radius of gyration of the \( P(R) \) function. Because the PDDFs are derived from the full range of scatter curves, rather than just the Guinier regions (36), and because the subtraction level for incoherent scattering and the minimum \( Q \) \( (Q_{\text{min}}) \) are properly determined, the data are not influenced by aggregation and other nonideal effects. The procedures developed by Ibel and Stuhrmann (30) have been used in analysis of the neutron scatter data of hyperacetylated and control core particles.

RESULTS

Particle Polyacrylamide Gels—Fig. 1 shows the polyacrylamide gel of the nucleosome particles fractionated according to their levels of acetylation. As previously reported by Bode et al. (26) the nucleosome particles show increasing retardation and dispersity with increasing acetylation. It has been suggested that this effect may result from a combination of nucleosome unfolding and DNA length polydispersity. The DNA lengths given in Table II for the high, medium, and low states of acetylation (method 1) are sufficiently similar that differences in DNA lengths between the fractionated acetylated particles are unlikely to be a major factor in the gel behaviors of Fig. 1. The gel behavior results from an increase in anisotropy of the nucleosome particle with increase in acetylation. This increase in anisotropy can have several origins: (i) based on the effect of acetylation on the interaction of the histone H4 peptide-(1-23) with DNA (21) acetylation would be expected to release the N-terminal domains of H3 and H4 from DNA in the core particle. These N-terminal arms which extend for 25-30 residues are nonstructured when free and would be expected to increase the frictional ratio of the core particle. The effect of release of the N-terminal arms would be additional to: (ii) an unfolding of the nucleosome; (iii) release of the DNA ends entering and leaving the nucleosome; (iv) an opening of the nucleosome into two halves about a central hinge; and (v) a sliding of the histone octamer on the DNA of the nucleosome giving one free DNA end of varying length. Neutron scatter studies of hyperacetylated particles allow a test of some of the above possible effects, particularly the unfolding of the nucleosome and the unwinding of long DNA ends.

Raw Data and Fit of Fourier-transformed Spline Functions—Neutron-scattering curves were obtained from hyperacetylated core particles and controls, both high and low states of acetylation from method 1 (Table I), in six different mixtures of \(^1\)H\textsubscript{2}O and \(^2\)H\textsubscript{2}O: 0, 23.5, 40.8, 68.5, 83.5, and 98.3% of \(^2\)H\textsubscript{2}O. Those given in Fig. 2 are for the most highly acetylated core particles. Momentum transfer values of data points which exhibited negative cross-section values due to statistical deviations are indicated by arrows. The scales of subsequent scatter curves from buffers of increasing proportions of \(^2\)H\textsubscript{2}O were each reduced by one decade. In some of the scattering curves, 0, 20, and 100% \(^2\)H\textsubscript{2}O solutions, small effects of interparticle interference were observed below \( Q = 0.24 \) nm\(^{-1}\). However, data below 0.24 nm\(^{-1}\) were not necessary for the indirect Fourier transform analysis which uses information

\(^1\)The abbreviation used is: PDDF, pair distance distribution function.
from the whole scattering curve and is not dependent on the low Q part of the Guinier region (37). The validity of this procedure is confirmed by the excellent fit to low Q of the curves determined by the indirect Fourier transform procedure to the scattering curves for the 40.8, 68.5, and 83.5% \( \text{H}_2\text{O} \) solutions. These low contrast scatter curves do not show effects of interparticle interference, and we draw attention to their importance in this analysis. A further check on these procedures is the consistency in the determination of molecular weight of the nucleosome particle from the data at all contrasts.

Neutron scattering requires comparatively high concentrations of particles. High concentrations of particles affect mostly the scattering at very low momentum transfer, i.e. the large distance part of the basic pair distance distribution function due to the shape of the particle. Our data analysis and interpretations emphasize changes in the pair distance distribution function at low contrasts. Such changes relate to the basic pair distance distribution function of the internal structure and are insensitive to high concentrations. Also, as pointed out, no interparticle effects are observed for the low contrast scatter curves.

Pair Distance Distribution Functions—12 equally spaced spline functions have been set up between zero and a maximum value of 13 nm, and the 59 data points of each diffraction curve were used to refine 12 spline height coefficients by the method of Glatter (36). The variations of the splines were further constrained by a stabilization parameter, i.e. Lagrange multiplier of 10^6. The solid lines in Fig. 3 are the pair distance distribution functions from the hyperacetylated core particles at positive contrasts, i.e. at scattering length densities of the particle which are smaller than the mean scattering length density of the solvent. This is for buffers with \( \text{H}_2\text{O} \) contents less than the contrast matched point (see below); the broken lines are from scatter curves at negative contrast, i.e. at scattering length densities of the solvent exceeding the mean scattering length density of the particle. This is for buffers with \( \text{H}_2\text{O} \) content more than in the contrast matched position. The dot-dash curve at the bottom of Fig. 3 is the distance distribution function at the contrast matched position, i.e. zero contrast as calculated from the fundamental diffraction cross-section (see below). The pair distance distribution functions go to zero at the maximum chord length in the particle which, for all contrasts, is between 11.5 and 12.0 nm. The pair distance distribution function for 40.8% \( \text{H}_2\text{O} \) is mostly due to the DNA component whereas that at 68.8% \( \text{H}_2\text{O} \) is mostly due to the protein component. The 40.8% \( \text{H}_2\text{O} \) pair distance distribution function would be the most sensitive to the unwinding of DNA ends of the nucleosome particle pro-

### Table I

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<th>Acetylation level</th>
<th>Method 1</th>
<th>Method 2</th>
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<td>Number of acetyl/H4 groups</td>
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### Table II

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<td>Load</td>
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<td>Peak</td>
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<tr>
<td>Trailing</td>
<td>199</td>
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**Fig. 2.** Neutron-scattering cross-section/unit solid angle in \( b=10^{-12} \text{ cm}^2/\text{sr} \) of hyperacetylated nucleosomal particles in buffers of variable heavy water content. The different volume fractions of \( \text{H}_2\text{O} \) were: 0% (Δ, upper curve scale 1:1); 23.5% (●, scale 1:10); 40.8% (●, scale 1:100); 68.8% (●, scale 1:1,000); 83.5% (○, scale 1:10,000); and 98.3% (●, scale 1:100,000). Momentum transfer values of data points exhibiting negative cross-section values due to statistical deviations are indicated by arrows. Data points of scattering curves obtained at high contrasts, i.e. 0 and 100% \( \text{H}_2\text{O} \) and below Q/0.24 nm\(^{-1} \) (thin vertical line) were affected by interparticle interferences. They were not used in the data analysis. The solid lines are fits to the data points from Fourier-transformed pair distribution functions according to the method of Glatter (36).
have been reported for core particles with 146 bp base pairs of DNA (2). From the scattering length density of the nucleosome particle $\rho_{\text{cm}}$, its dry volume $V_f$ can be obtained from

$$\rho_{\text{cm}} = \sum \frac{b_i}{N_r},$$

where $\sum b_i$ is the sum of the scattering lengths of the constituent atoms of the nucleosome particle from its composition and knowing the fraction of labile protons (1, 2). This gives $V_f = 233.1 \text{ nm}^3$. $V_f$ is also the sum of the partial specific volumes of the histones in the octamer (139.6 nm$^3$) and the 162 base pairs of DNA (90.7 nm$^3$) which gives an estimated $V_f = 230.2 \text{ nm}^3$. The close agreement of the measured $V_f$ based on the zero angle scatter intensities with the estimated $V_f$ from the partial specific volumes demonstrates that all of the hyperacetylated nucleosome particle is contributing to the scatter curves and the pair distance distribution functions.

The slope of the straight line gives an invariant volume, $V_r$, of the hyperacetylated core particle of $172 \pm 10 \text{ nm}^3$. In neutron scattering employing contrast variation by buffers of different volume fractions of heavy water, the invariant volume (38) excludes both the volume occupied by loosely bound water molecules and a volume around each labile proton site corresponding to half the volume of one water molecule. The relative distance of the data points from the straight line along the ordinate was always less than 3% with the exception of the point at 40.8% $^3\text{H}_2\text{O}$ which was 27% too high. Note that if $R_g$ DNA had been underestimated a low value of $I_0$ would be expected at 40.8% of $^3\text{H}_2\text{O}$.

Contrast Dependence of the Radius of Gyration—The radius of gyration for the hyperacetylated core particles were calculated from the second moment of the pair distribution function and their accuracy estimated from values obtained from different runs using different stabilization parameters. The Stuhrmann plot (29, 30) of $R_g^2$ versus $1/\rho$ is given in Fig. 5 (heavy line); $\rho$ is the contrast, i.e. the difference between mean scattering length density of the particle and the scattering length density of the solvent). The thin line is the Stuhrmann plot obtained for the larger chicken erythrocyte nucleosome particles obtained by Hjelm et al. (1), and the dashed line shows the more recent data obtained for core particles by Sibbet et al. (39). A third set of data obtained earlier for core particles gives a line with a lower slope (2).
The radius of gyration at infinite contrast that of the protein moiety and that of the DNA moiety have been calculated from the Sturmann plot (Table III) and are compared with the $R_g$ values obtained from previous studies (1, 2, 4). As can be judged, within the errors of measurement, they are indistinguishable from those of the native bulk core and reconstituted particles.

**Fundamental Scatter Cross-sections—**As previously described (1), the fundamental scatter cross-sections were obtained by fitting parabolas to the best curve fits to the neutron scatter data of Fig. 2 (solid lines) after normalization to the straight line of Fig. 4.

$$
\frac{d\sigma}{d\Omega} (Q) = \rho^{-2} \left( \frac{d\sigma}{d\Omega} (Q) \right)_C + \rho \left( \frac{d\sigma}{d\Omega} (Q) \right)_{CS} + \left( \frac{d\sigma}{d\Omega} (Q) \right)_S
$$

They are given in Fig. 6 and are indistinguishable from the fundamental scatter cross-sections obtained for native bulk core particles (2) and for chicken erythrocyte nucleosome particles. The scatter cross-section due to the shape of the invariant volume accords with a flat disc 11.0 nm in diameter and 5.5–6.0 nm in thickness as described previously (1, 2). It does not accord with a globular structure for a nucleosome (8), providing that this structure is maintained in the excised core particle and no structure rearrangement occurs. The fundamental scatter curves allow the scatter curve of the hyperacetylated core particle at any contrast to be calculated.

<table>
<thead>
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<th>TABLE III</th>
<th>Radii of gyration</th>
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<td>Hyperacetylated nucleosome particles</td>
<td>Chicken erythrocyte nucleosome particles (2)</td>
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<tr>
<td>$R_{c(DNA)}$</td>
<td>$R_{c(core)}$</td>
</tr>
<tr>
<td>nm</td>
<td>nm</td>
</tr>
<tr>
<td>4.75</td>
<td>4.06</td>
</tr>
<tr>
<td>4.75</td>
<td>4.04</td>
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<tr>
<td>4.8</td>
<td>3.92</td>
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</table>

They calculated scatter curves have much smaller statistical deviations and systematic errors because they derive from all the neutron scatter data. Scatter curves have been calculated for zero contrast; for the contrast matched position at 40% $^2$H$_2$O when the protein component is contrast matched and the DNA component dominates the scatter; and for that at 65% $^2$H$_2$O when the DNA is contrast matched and the protein component dominates the scatter. These more accurate scatter curves can be used to obtain accurate distance distribution functions for the protein component (i.e. at 65% $^2$H$_2$O), for the DNA component (i.e. at 40% $^2$H$_2$O), and for the contrast matched position (i.e. at 0.2964 $\times$ 10$^{-6}$ cm equivalent to 50.7% $^2$H$_2$O:49.3% $^1$H$_2$O). These pair distance distribution functions are given in Fig. 7.

$HC$ is the pair distance distribution function of the histone core. Most of the vectors can be drawn in the central core of the histone octamer (H2A, H2B, H3, H4), which has a dimension of 7.0 $\times$ 5.6 nm (5). However, the histone core distance distribution function does not go to zero at 7.0 nm but extends out to 12 nm. About 15% of the vectors are contained in the vector region between 7 to 12 nm and could come from regions of the histone octamer which extend out from the central histone core. Fluctuations in the density of DNA about the contrast-matched solvent density may also contribute to this region. The pair distance distribution function for DNA shows a pronounced minimum centered at about 5.7 nm. This results from the colliding of the 1.7 $\pm$ 0.1 turns of DNA on the periphery of the core particle and the absence of “DNA” vectors in the central region occupied by the histone core. The third pair distance distribution function is from the scatter curve of the particle at the contrast matched position which results from the internal structure fluctuations in the particle. The negative values at distances $R$ of half the maximum distance within the particle indicate a centrosymmetric arrangement of regions of opposite sign in contrast $\rho$. Concerning the shape of the histone core neutron scatter studies have given its $R_g$ of 3.3 nm and its PDDF, $P(R)$ curve, i.e. curve $HC$ in Fig. 7. If all of the core histones were contained within the shape of the histone core of 7.0 $\times$ 7.0 $\times$ 5.7 nm from the core particle crystal structure (9), then its $R_g$ would be 2.55 nm. The measured histone $R_g$ of 3.3 nm led to an earlier proposal that about 20% of the histone molecules, probably the N-terminal domains, were external to a complex of the globular domains of the core histones. This would also account for the tail of vectors between 7.0 and 12.0 nm in the $HC$ $P(R)$ curve of Fig. 7. This is in accord with the findings: (i) that the N-terminal regions of H2A and H2B are not bound within the core particle and the N-terminal domains of H3 and H4 can be released by 0.6 M NaCl without the core particle unfolding (40); and (ii) that proteolysis of the core particles selectively digest the N-terminal domains also with-

**Fig. 6.** The three fundamental scatter cross-sections. Top, contrast-independent scatter cross-section, which is due to fluctuations of scattering length density within the particle around its mean value. The corresponding pair distance distribution function is shown in Figs. 3 and 7. Bottom, quadratic term in the scatter, which is due to the shape of the invariant volume “occupied by the particle.” The scatter measured at 0% $^2$H$_2$O or 100% $^1$H$_2$O is due largely to this function. The units are chosen such that multiplication with the square of the difference in volume fraction of $^2$H$_2$O, i.e. $(X - 0.507)^2$ which is equal to $(\rho(\rho^2)H_2O - \rho_H2O)$, yields the value of the cross-section term $(d\sigma/d\Omega)Q^2$ in units of $\text{bsr}^{-1}$.

**Fig. 7.** The distance distribution functions (in $10^{-13}$ cm units) of scattering curves derived from the fundamental cross-sections for $X = 0.40$ (DNA); for $X = 0.65$ ($HC$); and for $X = 0.507$ (--- --- line).
out unfolding the particle (22-24).

In Fig. 8, the observed \( P(R) \) curve for the histone octamer is compared with calculated \( P(R) \) curves for (i) a sphere of radius 4.25 nm which gives a \( R_g \) of 3.3 nm; (ii) an oblate ellipsoid 7.0 \( \times \) 7.0 \( \times \) 5.7 nm from the histone component of the core particle crystal structure (5); and (iii) a prolate ellipsoid of 11 \( \times \) 6.5 \( \times \) 6.5 nm approximately the shape of the histone octamer from its crystal structure (8). Clearly the \( P(R) \) curve for the sphere does not fit the experimental \( P(R) \) curve \( HC \). As expected the calculated \( P(R) \) curve for the oblate ellipsoid does not fit the observed curve for the long vectors out to 12 nm. However, if 20% of the histones, the N-terminal domains, were located between 7.0 and 12.0 nm a much better fit would be expected. The prolate ellipsoid, 11 \( \times \) 6.5 \( \times \) 6.5 nm, has vectors out to 11 nm and gives a good fit to the observed \( P(R) \) curve which would also be improved if some of the N-terminal domains were external to the ellipsoid.

**Neutron Scatter Studies of Medium and Low Acetylated Core Particles**—The neutron scatter curves, pair distance distribution functions, fundamental scatter functions, and Stuhrmann plots (data not shown) of the low acetylated core particles were the same, within experimental error, as those reported above for the most highly acetylated core particles.

**DISCUSSION**

It has been reported (26) that when the level of acetylation of a core particle is 10 or more acetyl groups heterogeneity is observed in nondenaturing gel electrophoresis as is also shown in Fig. 1. An increased frictional resistance was observed at the higher levels of acetylation which was attributed to an increased conformational freedom of the particles, i.e. an opening of the particles. Electron micrographs of fractionated acetylated core particles showed heterogeneity in the most slowly migrating particles with 37% of these particles completely or partially unfolded and the remaining particles having diameters of 25 nm instead of 11 nm (27). For the next most highly acetylated fraction, the fraction of unfolded particles was 21%. Such structural changes in solution would be easily detected by neutron scatter techniques. For example, a nucleosome core particle transition at about 1.5 mM NaCl observed by hydrodynamic methods (41-43), fluorescence (44-46), and electrobirefringence (47-49) has been studied by neutron scatter techniques and shown to be due to a partial unwinding of the DNA with little effect on the histone octamer core (50). The highest level of acetylation of core particles used in this neutron scatter study is 2.4 acetates for the H4 molecules which correspond to the highest level reported for H4 in the study of mobility in gel electrophoresis (26, 27), and they demonstrate the same gel heterogeneity (Fig. 1).

Neutron scatter studies in effect provide a "snapshot" picture of the distribution of conformational states of the hyperacetylated core particles in solution. Thus, if high levels of acetylation result in an unfolding of the core particle, this should be easily detected by comparing the neutron scatter parameters of the hyperacetylated core particles with those of bulk core particles. In particular, the parameter \( R_g(DNA) \) is a sensitive indication of any unfolding of the core particle. There was no change in \( R_g(DNA) \) for the samples studied here, and Table III shows that \( R_g(DNA) \) of the hyperacetylated particles is identical to the \( R_g(DNA) \) values obtained for the previously studied bulk core particles (1, 2, 39). Further, the fundamental scatter functions and the \( ^2H_2O \) distance distribution functions are very similar to those obtained for bulk particles (1, 2, 39). From these results it can be stated that there is no detectable unfolding of the hyperacetylated particles free in solution.

Because the states of acetylation of the core histone will be distributed about the average values of 2.4 acetates/H4 molecule there will be a proportion of the core particles with even higher states of acetylation. The accuracy of the neutron scatter data is such that the unfolding of a small proportion of core particles (10%) would be expected to give a measurable change in \( R_g(DNA) \). Therefore, it seems unlikely that at even higher levels of acetylation that unfolding of the nucleosome occurs though this remains to be shown by studies of fully acetylated core particles. Such findings are in accord with thermal denaturation studies of hyperacetylated mono-, di-, and trinucleosomes which showed that acetylation resulted in a small but reproducible reduction in the major melting transitions (20). This was attributed to the binding of the N-terminal domains of the core histones to the DNA at the entry and exit to the nucleosome and the release of these domains by acetylation resulting in a small thermal destabilization of the central DNA region. Acetylation of the core histones would, therefore, be expected to have only a small effect on the overall stability of the core particle. Both the pair distance distribution functions (Fig. 3) and the determination of \( R_g(protein) \) and \( R_g(DNA) \) (Fig. 4) show that unfolding of the hyperacetylated nucleosomes of a significant proportion of these nucleosomes can be ruled out.

The question of whether there is an unwinding of the DNA ends or a change in the direction of the DNA ends of the particle is more difficult to ascertain. This is because a nucleosome particle with free DNA ends will have only a small fraction of its scattering density outside of a largely nucleosomal particle, particularly at high contrasts. Überbacher et al. (50) have addressed this problem in their neutron scatter study of nucleosome structure at low ionic strength. In their studies at high contrast in \( ^2H_2O \) and \( ^3H_2O \) they show the effect of very low ionic strength on the Stuhrmann plots and pair distance distribution functions of 146- and 175-bp nucleosome particles. In particular the pair distance distribution function which goes to zero at 12.5 nm for the compact form of the nucleosome particle extends to 20 nm and above for the nucleosome particle at very low ionic strength which is interpreted as an unwinding of long DNA ends. The data presented here for the hyperacetylated nucleosome particles do not show evidence for an unwinding of long DNA ends. This conclusion is based on the pair distance distribution function of the DNA moiety of the nucleosome particle constructed for 40% \( ^2H_2O \) (Fig. 7). At this contrast the histone core is matched. The total resultant scattering mass is that of the DNA, and its constructed pair distance distribution function would be sensitive to an unwinding of the DNA ends.
For comparison the directly determined pair distance distribution function for 33% $^2$H$_2$O, very close to the protein contrast matched conditions, is very similar to that constructed for the 40% $^2$H$_2$O solution. As for the other low contrast neutron scatter curves, the 35% $^2$H$_2$O solution curve does not show interparticle effects. Further, the measured dry volume, $V_d$, from the total neutron scatter data of 233.1 nm$^3$ is the same as that expected from the particle specific volumes of 230.3 nm$^3$, indicating that the neutron scatter from all the hyperacetylated particles has been taken into account and all of the DNA scatter is included in the analysis. A structural rearrangement of the core particle induced by acetylation and resulting in a change in direction of the DNA entering and leaving the nucleosome and thus affecting higher order structure would not be detected by this low resolution data.

Other more indirect experiments suggest that the basic N-terminal domains of the core histones have only a small effect on the structure of the nucleosome core particle. In the initial crystallographic study of core particle crystals it was reported that the core histones were proteolysed, and it appeared that a quarter of each molecule had been lost (23). It is probable, based on subsequent controlled proteolysis experiments (24), that the N-terminal domains had been lost. Further extensive trypsin digestion of histones in core particles is required before there is a marked unfolding of the core particle (22).

Thus, the compact nature of the core particle appears not to be markedly affected by the loss of the N-terminal domains by proteolysis. A possible reason for the difference between the neutron scatter studies and the gel electrophoresis and electron microscopic studies concerns the effect of acetylation on the dynamics of the acetylated particles. In the neutron scatter studies, the particles are free in solution whereas in electron microscopy, they would be subjected to large stresses in the sample preparation. Such stresses may affect the structures of hyperacetylated core particles more than the structures of the control core particles. Questions which cannot be addressed by studies of isolated nucleosomal particles concern the effects of histone acetylation on chromatin structure. With the current model of looped domains of chromatin fibers, DNA provides continuity which would be able to transmit the effects of structural changes at the nucleosome level around the chromatin loop. The constraints on individual nucleosomes provided by the DNA may result in acetylated nucleosomes unfolding more easily than unacetylated states. It has been suggested that core histone acetylation may destabilize the 34-nm supercoil of nucleosomes or solenoid (18, 19). Conformational studies of chromatin isolated from butyrate-treated cells have not revealed a marked effect of intermediate and mixed states of acetylation on the 34-nm supercoil (51).

It may be that structural effects are observed only at the highest levels of acetylation which would require fractionation of chromatin according to the levels of acetylation. Another complication is that butyrate treatment also induces high levels of a subfraction of the very lysine-rich histone, H1$^b$, and its effect on higher order structure stability is not known. In another less direct study the N-terminal regions of the core histone, which contain all the sites of acetylation, have been removed from H1-depleted chromatin and H1 added back. This core histone-trimmed chromatin was unable to form the 34-nm supercoil (24). This supports the earlier suggestion that the N-terminal domains of the core histones are also involved in stabilizing the 34-nm supercoil either by direct interaction with linker DNA or by providing the correct binding site for the globular central domain of H1 (18, 19). Further studies are required of fully acetylated core particles and of highly acetylated oligonucleosomes to elucidate the effect of acetylation on chromatin structure.

These and previous neutron scatter studies (1-4, 5, 28) have relevance to the recently proposed new model for the nucleosome based on the crystal structure of the histone octamer (8, 52, 53). Two turns of DNA of pitch 3-4 nm are coiled around the long axis of the prolate ellipsoid of 11.0 $\times$ 7.0 $\times$ 6.5 nm to give a nucleosome model of length 11.0 nm and diameter 10.5 nm which is approximately twice the thickness of the core particle structure from neutron scatter and x-ray diffraction (2-7). This neutron scatter analysis of the overall shape of the hyperacetylated core particle accords with the previous studies leading to the flat disc core particle structure. However, it has been suggested that this globular nucleosome structure exists in chromatin but not for the excised core particle which undergoes a structural contraction when the continuity of the DNA is lost (8). As discussed earlier, the shape of the histone core so far identified in the crystal structure of the core particle (5) gives a calculated $R_g$ of 2.55 nm compared to the measured $R_g$ of 3.3 nm (4). Its calculated $P(R)$ curve, as expected, does not fit the observed $P(R)$ curve at the long vectors (Fig. 8). To account for this difference and to accommodate the conformational behavior of the N-terminal domains of the core histones (40) and their selective proteolysis (24) about 20% of the histone mass corresponding to the N-terminal domains was located outside of the 7.0 $\times$ 5.7 nm histone core which would increase the $R_g$ of the histone core and put vectors in the region of 7.0-11.0 nm improving the fit to the experimental $P(R)$ curve. The prolate ellipsoid 11 $\times$ 6.5 $\times$ 6.5 nm of the isolated histone octamer (8) has vectors in the pair distance distribution function out to 11.0 nm and gives a better fit to the observed $P(R)$ curve which could also be improved at the higher vectors if N termini of the core histones were external to this shape. Thus, if no allowance is made for the location of the core histone N-terminal domains then the prolate ellipsoid (8) gives a better fit to the observed $P(R)$ curve than the oblate ellipsoid (5). If the N-terminal domains are located outside of the oblate ellipsoid then it would be difficult to distinguish this model from the prolate ellipsoid from neutron scatter analysis which gives spherical averages of the core particle structure. The best overall shape of the core particle from the neutron scatter data is a flat disc, 11.0 $\times$ 5.5-6.0 nm (2, 4), and this is in agreement with the shape of the core particle from the crystal structure (6). The proposal that the globular 11.0 $\times$ 10.5-nm nucleosome exists in chromatin but the disc-shaped core particle is an artifact of preparation can be tested by data already obtained from neutron scatter studies of extended chromatin at low ionic strength (28). These gave the mass/unit length of extended chromatin corresponding to 1 nucleosome/10-12 nm, the DNA cross-section $R_c$ of 3.4 nm, and the histone cross-section $R_h$ of 2.1 nm. To fit a linear array of flat disc-shaped nucleosomes to these parameters required the discs to be arranged with their faces parallel to the fibril axis, i.e. edge-to-edge (28). There is no arrangement of globular 11 $\times$ 10.5-nm nucleosomes which would give both of the cross-section $R_c$ values for DNA and histone. Also the fiber x-ray diffraction pattern is fully consistent with that expected from a supercoil of radially arranged nucleosomal flat discs (52).

Thus, there is strong evidence to support the flat disc nucleosome in bulk chromatin as well as in the isolated core particle. A possible explanation for the crystal structure of the histone octamer of a prolate ellipsoid 11.0 $\times$ 10.5 nm is that it is a polymorphic form of the octamer generated by the crystallization conditions and could represent an induced functional state of the octamer.
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