mal, perhaps for only a brief period each tidal cycle, as currents are decreasing on the approach to slack water. To cope, these *Pseudocalanus* have acquired the capacity to gorge whenever food is available.

How often the individual *Pseudocalanus* is in a favorable position to feed must still be determined. Although zooplankton concentrations near the ice may be large, the same species occurs throughout the water column. Whether animals at depth, through daily, seasonal, or ontogenetic migration, or through strong tidal mixing, can reach the vicinity of the epontic primary production is problematic. However, this potential source of nutrition can apparently extend the period of growth for some members of the calanoid copepod community.

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Shape Analysis of the Histone Octamer in Solution

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The conformation of the histone octamer is shown to depend upon the specific salt used to solubilize it. In 2*M* sodium chloride the octamer is similar in size and shape to the histone component of crystallized core nucleosomes. In contrast, in 3.5*M* ammonium sulfate the octamer is elongated, resembling an ellipsoid with approximate dimensions of 114 by 62 by 62 angstroms. These results indicate that the elongated conformation seen in the 3.3 angstroms electron density map of the histone octamer crystallized in ammonium sulfate is due to the particular salt conditions used.

THE FUNDAMENTAL SIZE, SHAPE, and DNA superhelical parameters of core nucleosomes, deduced from x-ray crystallographic studies (*1, 2*), are the cornerstones of virtually all theories concerning the manner in which DNA is packaged into chromatin. These attributes also provide the basis for studying, at the nucleosomal level, regulatory mechanisms whereby DNA accessibility is controlled during transcription and other functions.

Recently, a 3.3 Å resolution map of the histone octamer was reported by Burlingame *et al.* (*3*). The histone octamer structure they presented differed surprisingly in shape from that of the histone octamer in core nucleosomes as determined by the crystallographic studies, by neutron scattering (*4, 5*), and by electron microscopy (*6, 7*). Burlingame *et al.* suggested the alternative structure for the nucleosome based upon their placement of DNA, by inspection, on the octamer structure. The result was a particle significantly larger than a core nucleosome, and so different with respect to DNA superhelical parameters as to challenge many preceding ideas about chromatin structure.

Burlingame *et al.* addressed the discrepancy between the two types of structures by proposing that core nucleosomes are artifacts generated by removing H1 and linker DNA from chromatin, and concluded that crystallographic studies of core particles generate maps of structures that do not represent the native chromatin subunit. Klug *et al.* (*8*) raised the possibility that the crystallographic methods used in (*3*) were incorrect, and therefore the octamer described in (*3*) does not exist in solution. Uberbacher and Bunick suggested that the extended configuration of the crystallized octamer is attributable to the absence of DNA and the high concentration of ammonium sulfate in which the histones were crystallized (*9*).

Since small-angle neutron scattering (SANS) affords the opportunity to make direct measurements of the size and shape of biomolecules as they exist in solution, we performed a SANS study of the histone octamer in conditions similar to those employed by Burlingame *et al.* in order to answer three questions: Does the extended octamer exist in solution? If so, does its conformation reflect a salt effect? Finally, if it exists in solution, what is its relation to nucleosomes and to chromatosomes (nucleosomes retaining H1 and part of the linker DNA)?

Histone octamer, prepared as in (*10*), was examined by SANS in two sets of conditions: first, in 2*M* NaCl at *p*H 7.5; second, in 3.5*M* ammonium sulfate through a *p*H range of 5.8 to 7.5, a salt condition very similar to that used to crystallize the histone octamer (*3*). Octamer samples were prepared in H_2O to avoid any question that D_2O might affect conformation.

Neutron scattering measurements were performed on the 30-m small-angle neutron scattering instrument at the National Center for Small-Angle Scattering Research, Oak Ridge National Laboratory. The scattered neutrons were detected by a two-dimensional position-sensitive ^{3}He detector with 1-cm^2 elements (64 by 64). The sample detector distance was set to 3.0 m, which gave a usable K (scattering vector) range of from 0.012 to 0.15 Å^{-1}. All samples were maintained at 4°C throughout preparation and data collection. At least four independent measurements were made on each type of sample and buffer. A concentration series for each type of sample demonstrated the absence of concentration-dependent effects, such as aggregation or interparticle interference. This finding was further confirmed by the fact that different samples showed consistent ratios of forward scattering [$I(o)$] to concentration (c), and produced Guinier plots with no deviation from linearity in the

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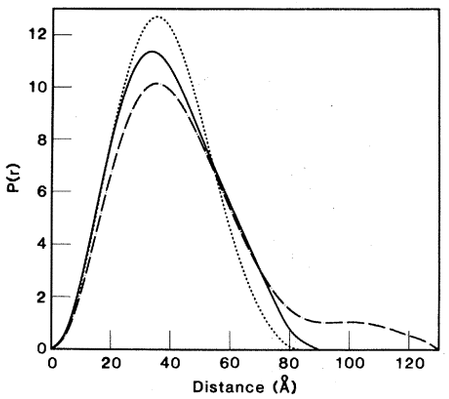

Fig. 1. Length distribution functions, $P(r)$, in arbitrary units, for the histone octamer in various experimental conditions. (A) · · ·, histone component of the core particle crystal structure (*2*); (B) —, octamer in 2*M* NaCl; (C) — · — · , octamer in 3.5*M* ammonium sulfate, *p*H 5.8 (other ammonium sulfate curves are similar).

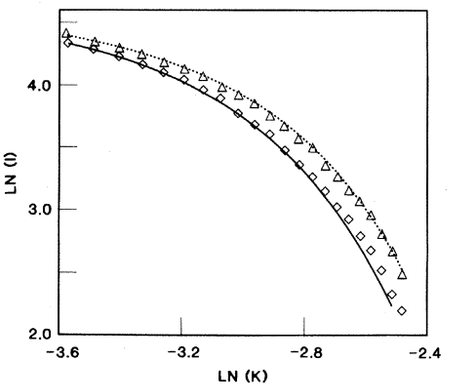

Fig. 2. Scattering curves for histone octamer in various conditions. (A) · · ·, histone component of the nucleosome core particle, calculated from the x-ray structure (*2*); (B) △△△, histone octamer in 2*M* NaCl, experimental scattering data; (C) ◇◇◇, histone octamer in 3.5*M* ammonium sulfate, *p*H 6.5, experimental scattering data; (D) —, curve calculated for 114 by 62 by 62 Å ellipsoid fit to (C).

low-angle region. Statistical errors were propagated throughout the analysis and are reflected in the final estimates of parameters.

In NaCl solution the radius of gyration of the octamer is approximately 30.0 Å, whereas it ranges from 32.1 to 32.7 Å in ammonium sulfate (Table 1). These values are larger than the 28.4 Å radius of gyration calculated from the histone portion of the x-ray crystal structure of the core nucleosome (*2*). In 3.5*M* ammonium sulfate the radii of gyration of the samples increase slightly as the *p*H is decreased (Table 1).

The length distribution function, $P(r)$ (Fig. 1), is a frequency distribution of all the point-to-point pair distances between scattering centers (atoms) in a molecule. The maximum distance for which the function is positive corresponds to the longest chord through the molecule. Examination of Table 1 and Fig. 1 reveals several striking features. First, the octamer in 2*M* NaCl is slightly larger than the octamer in the core particle crystal structure, although its shape, as judged from the shape of the length distribution function, is similar to that of the core particle octamer. The maximum chord length through the molecule in 2*M* NaCl is about 90 Å, compared to about 85 Å for the octamer in the core particle. Apparently, removal of the DNA from the octamer and neutralization of histone charges with NaCl result in an overall expansion of the octamer. Second, in 3.5*M* ammonium sulfate through the *p*H range of 5.8 to 7.5, the octamer appears significantly larger and more extended than it does in either 2*M* NaCl or core particles. The actual extent of the octamer and the amount of mass at the extremes of the molecule increase slightly in 3.5*M* ammonium sulfate as *p*H is decreased within the range examined. According to the length distribution function, the longest chord through the octamer in ammonium sulfate is at least 110 Å and may be as great as 130 Å.

To analyze the scattering data for the shape of the octamer, scattering was calculated for homogeneous ellipsoidal and cylindrical models. The radii of gyration of the models were maintained at the experimentally determined value while axial ratios were varied. We then compared these calculated scattering curves to the observed data to derive a best fit. In all cases ellipsoids yielded the best fits, so cylindrical fits will not be discussed. The best fit ellipsoids for the octamer in the various conditions are shown in Table 1. In all cases, the reduced χ^2 parameter for the best fit was less than 1.1.

We found (Fig. 2) that in 3.5*M* ammonium sulfate, within the *p*H range examined, the best geometric representation of the histone octamer is a prolate ellipsoid of approximately 114 by 62 by 62 Å, a dimension in good agreement with the results of Burlingame *et al.* The strong similarity between the scattering curve of histone octamer in 3.5*M* ammonium sulfate at *p*H 6.5 and the curve calculated for its best fit ellipsoid model means that in these conditions the octamer strongly resembles a homogeneous ellipsoid. This implies that considerable globular mass is extended, not merely a few histone projections representing a small fraction of the total mass of the octamer.

Since the ellipsoid is a smooth geometric representation of the octamer, which undoubtedly has an irregular surface, the maximum dimension of the ellipsoid does not necessarily model the maximum extent of the molecule. Indeed, the length distribution functions indicate that at least some chords through the octamer in 3.5*M* ammonium sulfate, through the *p*H range 5.8 to 7.5, exceed the ellipsoid dimensions. Consistent with this is the observation that in the octamer crystal structure (*3*) two small "tails" protrude perhaps 10 Å beyond the bulk of the molecule in the long axis direction of the octamer. The length distribution function analysis and best ellipsoidal approximations of the octamer in either 2*M* NaCl or core nucleosomes show that the maximum chord through these two structures is about 20 Å shorter than through their ammonium sulfate counterpart.

The apparent shape and extent of the histone octamer is clearly different in ammonium sulfate solution than in NaCl solution. This is not altogether surprising since the interactions between salts and macromolecules are complex (*11, 12*). Not only is charge shielding dependent upon the type of salt used, but different salts affect hydrogen bonding and the solubility of nonpolar groups to different degrees, and so have varying abilities to bring about protein conformation changes. Moreover, since salts penetrate the interstices of the histones, whereas DNA can only neutralize charges on the octamer surface, it is conceivable that replacing DNA with certain salts causes additional changes in the shape of the octamer.

A comparison of the compact histone octamer in core nucleosomes to the octamer structure of Burlingame *et al.* suggests the nature of their relationship. The most striking difference between the two structures is the presence of large (4 to 14 Å) solvent channels separating the H2A/H2B dimers and the $(H3/H4)_2$ tetramer in the octamer

Table 1. Size and shape of histone octamer in different solvent conditions.

Octamer solvent conditions	Radius of gyration (Å)	Dimensions of best fit ellipsoid (Å)*
In core nucleosome crystals†	28.4	92 by 62 by 62
2*M* NaCl, *p*H 7.5	30.0 ± 0.3	96 by 66 by 66
3.5*M* $(NH_4)_2SO_4$, *p*H 7.5	32.1 ± 0.2	110 by 66 by 66
3.5*M* $(NH_4)_2SO_4$, *p*H 6.5	32.3 ± 0.4	114 by 62 by 62
3.5*M* $(NH_4)_2SO_4$, *p*H 5.8	32.7 ± 0.3	120 by 60 by 60

*Error estimates on dimensions of best fit ellipsoids are less than ± 2 Å. †Calculated from the protein component of crystallized core nucleosomes (*2*).

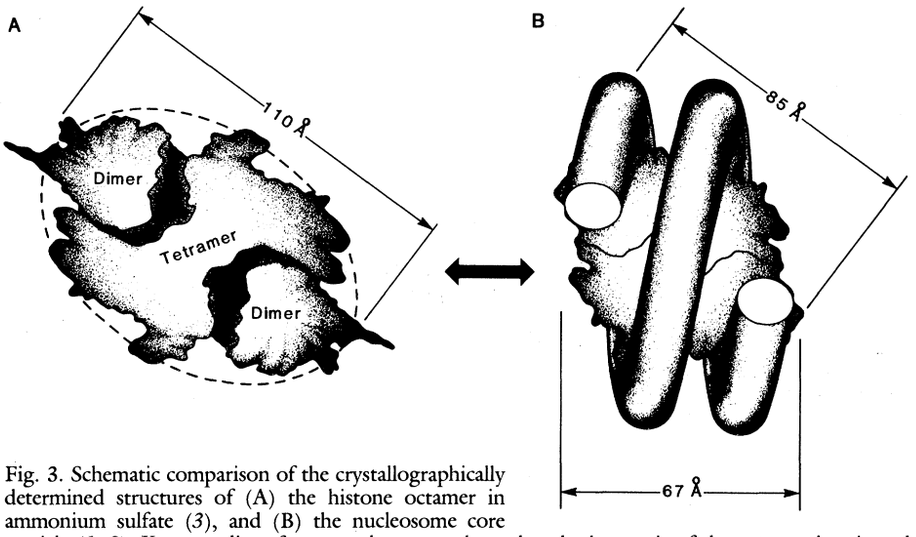

Fig. 3. Schematic comparison of the crystallographically determined structures of (A) the histone octamer in ammonium sulfate (*3*), and (B) the nucleosome core particle (*1, 2*). X-ray studies of core nucleosomes show that the long axis of the octamer is oriented almost 45 degrees to the DNA superhelical axis; (A) and (B) are arranged accordingly. Structure (A) is similar in size and shape to the octamer in 3.5*M* ammonium sulfate described in this study. Note the large solvent channels and the 20 Å "grooves" between the H2A/H2B dimers and the $(H3/H4)_2$ tetramer. In physiological solvent conditions and in the presence of DNA the histone subunits associate more tightly, as in (B).

crystallized in ammonium sulfate (*3*). The presence of these channels, and the 20 Å "grooves" between the dimers and the tetramer in the octamer crystals, corresponds with our observation that a significant amount of the mass of the octamer becomes extended in 3.5*M* ammonium sulfate, and may be evidence that the dimers are partially dissociated from the tetramer.

Since the histone octamer is substantially elongated in 3.5*M* ammonium sulfate as compared to its counterpart in core nucleosomes, the sites to which DNA would bind in physiological solvent conditions may not be obvious. If DNA binds extensively in the protein "grooves," as has been proposed (*3*), one might expect the accessibility of nucleosomal DNA to solvent to be noticeably impaired. McGhee and Felsenfeld (*13*), however, found that the major and minor grooves of nucleosomal DNA are nearly as accessible to solvent as is free DNA. In agreement with that study, core nucleosome structures (*1, 2, 4*) indicate that in most locations the DNA actually rides on the "high points" of the proteins. Consequently, we proposed (*9*) the relationship between the nucleosome core particle and the octamer in ammonium sulfate shown in Fig. 3.

From x-ray crystallographic studies of the nucleosome core particle, the long axis of the core octamer is oriented at about 45 degrees to the DNA superhelical axis (*1, 2*). Positioning 146 base pairs of DNA from corner-to-corner on the octamer crystallized in ammonium sulfate yields a hypothetical particle with an axial length of about 85 Å. If the H2A/H2B dimers associate more closely with the $(H3/H4)_2$ tetramer, as they likely do in the presence of DNA and at physiological ionic strength, the axial length of the resulting particle approaches 67 Å, the maximum particle thickness seen in core nucleosome crystals. Because of these considerations, the volume ratio of the octamer in ammonium sulfate to the octamer in core nucleosomes should not be 2:1 as originally stated (*3*), but much closer to unity. This indeed appears to be the case. The revised estimated volume of the extended octamer, 184,000 $Å^3$ (*8*), is only 25% larger than the calculated volume of the octamer in core nucleosomes, which is approximately 140,000 $Å^3$ (*14–16*).

The suggestion has been made (*3*) that the existence of this elongated octamer indicates substantial differences between core nucleosomes, from which the linker DNA and H1 have been removed, and the natural chromatin subunit. Because research on oligonucleosomes has yet to yield high-resolution details of nucleosome-nucleosome interactions, we are limited to studies of chromatosomes and H1-depleted chromatosomes as our best examples of native chromatin structure. If, in generating core nucleosomes for crystallization and other studies, the removal of H1 and the linker DNA leads to substantial reorganization of the inner histones, neutron scattering has not detected these differences. Uberbacher *et al.* (*17*) showed that, in nucleosomes containing linker DNA but depleted of H1, the histone octamer is the same size as the compact octamer in a nucleosome from which the linker has been removed. Chromatosomes contain both H1 and linker DNA; hence, they presumably resemble nucleosomes in situ more than do core particles. Neutron scattering studies indicate that the octamer component of chromatosomes is virtually identical in size to the octamer in nucleosome cores (*18*).

The most important implication of our study is that the octamer crystallized in ammonium sulfate (*3*) and the octamer in crystals of core nucleosomes (*1, 2*) are very closely related, and their seeming disparities are reconcilable. The radii of gyration of core nucleosome octamer and octamer in ammonium sulfate, and their respective length distribution functions, correspond suggestively to the major differences between the two x-ray structures. Since the histone octamer in 2*M* NaCl is shaped similarly to the octamer in core nucleosome crystals, extension of the long axis of the octamer in 3.5*M* ammonium sulfate must be attributed to a factor other than the removal of DNA. Our study demonstrates that the elongated 3.5*M* ammonium sulfate octamer is the result of a specific salt effect, and, as such, does not provide grounds for postulating an entirely different nucleosome structure.

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