Rapid mechanical changes have been demonstrated during excitation of almost all the excitable tissues examined. Therefore, the mechanical responses observed in the invertebrate retinas appear to be an expression of general physicochemical processes involving the plasma membrane and cytoskeleton of excitable cells.

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Crystals of the Octameric Histone Core of the Nucleosome

Abstract. *The undegraded core histone octamer has been crystallized in a form suitable for x-ray analysis. The hexagonal bipyramidal crystals reproducibly grow larger than 1.0 by 0.6 millimeter. X-ray reflections are observed from Bragg planes with spacings larger than 3.5 angstroms. The crystals have the symmetry of the space group $P3_121$ or its enantiomorph. There appears to be one histone octamer per asymmetric unit.*

Most of the DNA in eukaryotic chromosomes is organized into repeating structural units by its interaction with a set of basic proteins called histones (*1*). Electron microscopy of chromatin allows visualization of a fiber (100 Å diameter) with a repeating structure that resembles a string of beads (*2*). Brief digestion of chromatin with micrococcal nuclease yields nucleosomes containing about 200 base pairs (bp) of DNA, two each of the four core histone molecules H2A, H2B, H3, and H4, and one molecule of histone H1 (*3*). A series of nucleosomes corresponds to the repeating structures observed in electron micrographs. Further digestion of chromatin with micrococcal nuclease cleaves the less tightly complexed DNA and releases H1 and yields the nucleosome core particle, which consists of 146 bp of DNA wrapped around the octameric histone core $(H2A, H2B, H3, H4)_2$.

Extensive analysis of nucleosome core particles and their octameric histone core by electron microscopy (*4, 5*), x-ray (*6*) and neutron diffraction (*7*), and chemical cross-linking (*8*) has yielded a picture of the nucleosome core particle to a resolution of about 25 Å. It appears to be a wedge-shaped disk 110 Å in diameter and 55 Å at its thickest edge. One-and-three-quarter turns of double-stranded DNA are wrapped around the outside of the histone octamer in a superhelix with a pitch of about 28 Å. The octamer is also a wedge-shaped disk, about 70 Å in diameter and 55 Å high, possibly with a protruding ramp corresponding to the path of the DNA supercoil (*5*). To the resolution of the methods used, there is a twofold axis of symmetry in both the nucleosome core particle and the histone octamer.

The histone octamer can be extracted from chromatin as a stable undissociated particle in 2*M* NaCl (*9, 10*). In physiologic ionic strengths in the absence of DNA, the octamer dissociates into subunits: the $(H3, H4)_2$ tetramer and two (H2A, H2B) dimers (*10*). By combining the histone octamer or a mixture of the dimer and tetramer subunits with DNA under appropriate conditions, complexes that appear identical to nucleosome core particles by a number of criteria can be reconstituted (*11*). We have attempted to crystallize the $(H2A, H2B, H3, H4)_2$ histone octamer and its physiological subunits, the (H2A, H2B) dimer and the $(H3, H4)_2$ tetramer. We have previously reported our results with crystals of one subassembly of the core histones, the slightly proteolyzed $(H3, H4)_2$ tetramer from calf thymus (*12*). We report here the first crystals of the complete assembly of the undegraded histone octamer.

Chicken erythrocytes were obtained from Pel Freeze Biologicals. Nuclei were isolated be the method of Olins *et al.* (*13*). Histone octamer was isolated according to Eickbush and Moudrianakis (*10*). To prepare the octamer for crystallization, a concentrated (20 mg/ml) solution of pure chicken erythrocyte octamer in 2*M* NaCl, 1 m*M* EDTA, and 10 m*M* tris, *p*H 7.5, was dialyzed against 60 percent stock ammonium sulfate (70 g plus 100 ml of water), with 10 m*M* NaP_2O_7, *p*H 6.5, 5 m*M* EDTA, and 1 percent 2-mercaptoethanol (crystallization buffer). The next day this material was dialyzed against a 65 percent stock solution of ammonium sulfate and crystallization buffer. The following day the solution was clarified by centrifugation, and the protein in solution was placed in Zeppezaur tubes (*14*) and dialyzed against 67 percent stock ammonium sulfate and crystallization buffer. Crystals reproducibly grew in about 1 week and continued to grow for about 2 months. Increasing the concentration of the ammonium sulfate to 69 percent stock caused the crystals to continue to increase in size. All steps were carried out at 4°C.

The crystals are clear, nonbirefringent, hexagonal bipyramids (Fig. 1A) that grow larger than 1.0 by 0.6 mm. The

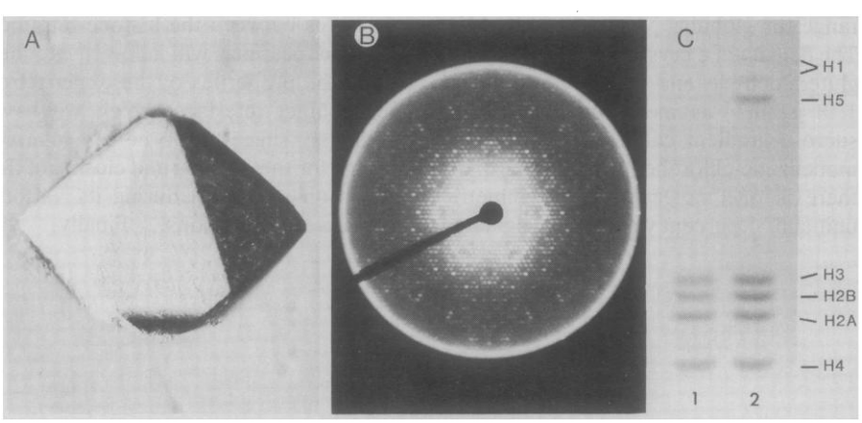

Fig. 1. (A) Light micrograph of a hexagonal bipyramidal crystal of the histone octamer that is about 0.4 by 0.5 mm. (B) A 12.5° screened precession picture of the zero layer looking down the threefold axis. Reflections at 3.5 Å resolution are apparent. (C) Photograph of sodium dodecyl sulfate–polyacrylamide gels (*10*); (lane 1) proteins recovered from a single crystal; (lane 2) acid-extracted chick erythrocyte total histones.

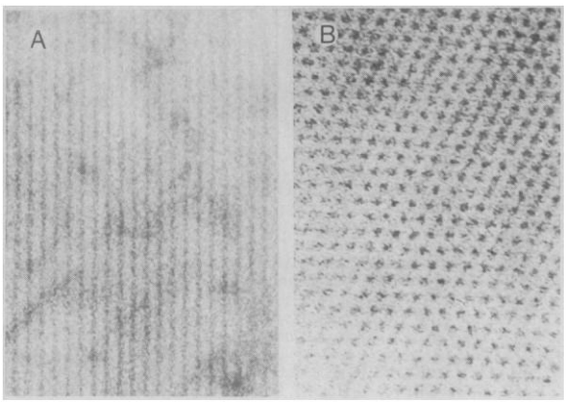

Fig. 2. Electron micrographs of thin sections of glutaraldehyde-fixed, Epon-embedded octamer crystals. The sections were stained by an ethanolic (50 percent) uranyl acetate solution and photographed at an instrumental magnification of ×150,000. (A) A section cut perpendicular to the *a* (or *b*) axis of the crystal. The narrow dark bands running through the micrograph are uranyl acetate–filled channels, and the broad light bands are columns of histone octamers. Preliminary optical diffraction of the micrographs revealed no order parallel to the columns. (B) A section cut perpendicular to the *c* axis. The projection of one unit cell is the parallelogram formed by connecting four of the dark channels. In the crystal, this is 119 Å, while in the micrograph it is about 95 Å, which corresponds to a 20 percent shrinkage caused by the sample preparation and examination.

gels from sodium dodecyl sulfate–polyacrylamide electrophoresis of the best crystals show that there is little or no detectable proteolysis of the four core histones, and that all the histones are present in equimolar amounts (Fig. 1C). X-ray reflections are observed from Bragg planes with spacings greater than 3.5 Å (Fig. 1B), and the intensity of the reflections decays less than 20 percent after 1 week in the x-ray beam. The crystals have the symmetry of the space group $P3_121$ (or its enantiomorph) with six asymmetric units per unit cell. The lattice constants are $a = b = 119$ Å, and $c = 103$ Å. The volume of the asymmetric unit is thus 2.1×10^5 Å^3. The chicken erythrocyte octamer has a molecular size of 108,000 daltons (*1*). If there is one histone octamer per asymmetric unit, then the V_M is 1.95 as calculated by the method of Matthews (*15*). This is within the observed range for globular proteins and indicates dense packing of the protein within the crystal. If there is one-half octamer per asymmetric unit, the V_M is 3.9, which is outside the observed range for globular protein crystals (*15*). The density of a crystal cross-linked with gluteraldehyde and soaked in water is 1.18 g cm^{-3} as measured in a Ficoll-sucrose gradient calibrated with a bromobenzene-chlorobenzene mixture. If there is one molecule per asymmetric unit and 35 percent water, the calculated density of the crystal is 1.20 g cm^{-3}. If there is one-half molecule per asymmetric unit and 67 percent water, the calculated density is 1.11 g cm^{-3}. The measured density thus supports the above calculation that there is one octamer per asymmetric unit. Also, electron micrographs of negatively stained thin sections of the crystal show a large unstained area (Fig. 2A and 2B), consistent with dense protein packing in the crystal. We have prepared a number of putative heavy atom derivatives and are in the process of analyzing one mercury and one platinum derivative as the first step in determining the structure of the histone octamer by multiple heavy atom isomorphous replacement.

The crystals of the histone octamer described here offer the potential for solving the structure of this important protein assembly at high resolution and independent of any limitations that could be imposed by the DNA base sequence heterogeneity present in the crystals of the nucleosomal core particle. Furthermore, the comparison of the crystal structures between the histone tetramer (*12*) and octamer will allow us to describe the properties of the dimer-tetramer contact interface which we have previously suggested to be a regulatory surface for the opening and closing of the nucleosome (*10, 16*) during its various functional transitions. Finally, the stereochemistry of the surface of the histone octamer at the resolution afforded by the crystals at hand should allow us to deduce the path of the DNA helix around the protein core by means of model building, as has been done for other DNA-binding proteins (*17*).

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