Tubular arrays of the actin–DNase I complex induced by gadolinium

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ABSTRACT We describe the preparation and structural analysis of ordered tubular arrays of the actin–DNase I complex. These structures consist of helically stacked rings; each ring is 73 Å thick, has a 240 Å outer and a 120 Å inner diameter, and has 7-fold rotational symmetry. The actin–DNase I complex forms tubes under conditions in which actin alone aggregates into crystalline sheets—i.e., in the presence of the trivalent cation gadolinium. Moreover, upon addition of an equimolar amount of DNase I, crystalline actin sheets are slowly converted to tubes. The tubes making the tubes contain a radial dyad axis that may be identical to the dyad axis of the unit cell of the crystalline actin sheet. Evidence is presented for this identification, which in turn allows tentative assignment of actin- and DNase I-containing regions in three-dimensional reconstructions of the rings. The structural analysis presented here may be useful in aligning available three-dimensional molecular models of actin determined from crystals of the actin–DNase I complex and from crystalline actin sheets with each other and ultimately within the biologically important actin filament.

Most actin-binding proteins interact specifically with filamentous actin, whereas at least two—DNase I and profilin—interact preferentially with monomeric actin. DNase I forms a tight 1:1 complex with actin, in which both the enzymatic activity of the DNase I and the ability of the actin to polymerize are inhibited (1–3). Although the actin–DNase I complex has been shown to exist in vivo (4), its functional role is still speculative. Nevertheless, the actin–DNase I interaction has proven to be useful—e.g., in quantitating the amount of unpolymerized actin in nonmuscle cells (5) and in probing the mechanism of actin filament depolymerization (6).

Three-dimensional crystals of the actin–DNase I complex have been obtained and subsequently used to determine its three-dimensional structure by x-ray crystallographic methods to ~5 Å resolution (7, 8). Although identification of the actin- and DNase I-containing portions of the composite electron density map has been attempted, assignment of intermolecular boundaries is often ambiguous at this resolution. To this end, the availability of a 2.5 Å map of DNase I alone (D. Suck, Ch. Oefner, and W. Kabsch, personal communication) should yield more definitive boundaries of the actin subunit within the actin–DNase I map. A molecular model of actin alone has been obtained to about 15 Å resolution by using electron microscopy and image processing of negatively stained two-dimensional crystalline sheets of pure actin (9), which can be induced by the trivalent lanthanide gadolinium (10–12). All of the presently available molecular models of actin consist of elongated, bilobed structures with similar overall shapes and dimensions.

Because none of these crystalline forms of actin appear to be constructed directly from actin filaments (7, 8, 9, 12), the resulting models of the actin subunit do not automatically allow one to build a molecular model of the functionally important actin filament (7, 9, 13). Present understanding of actin filament structure is based in large part on electron microscopy and image reconstruction of "negative stain replicas" of actin filaments, which at best yield consistent structural features to 30 Å resolution (9, 13–19). Unfortunately, this is insufficient to achieve unambiguous alignment of molecular models of the actin subunit within the resulting filament models (e.g., see refs. 7, 9, and 13). What is needed in a first instance are higher resolution and more consistent actual filament reconstructions and not higher resolution actin subunit models.

Here we report the induction by gadolinium of a previously undescribed type of ordered tubular array from a 1:1 mixture of actin and DNase I. We have analyzed the structure of these "tubes" using electron microscopy, three-dimensional reconstruction, and chemical crosslinking and have found that the symmetry of the unit cell is distinct from that of the three-dimensional crystals of the complex but is related to that of the crystalline pure actin sheets—i.e., both gadolinium-induced structures probably contain the same type of actin dimer. The use of these data in the determination of the relative orientations of the molecular models of actin derived from the different types of crystalline structures is discussed.

MATERIALS AND METHODS

Proteins. Acanthamoeba and rabbit skeletal muscle actin were purified as described by Aebi et al. (12). Bovine pancreatic DNase I (type I, Sigma) was dissolved in low ionic strength buffer at 5 mg/ml and used without further purification. Actin filaments were prepared by incubating 1 mg of muscle or nonmuscle actin per ml in 2 mM MgCl₂/50 mM KCl for 30 min at room temperature. The formation of crystalline actin sheets of rabbit muscle or Acanthamoeba actin was induced as outlined (12).

Crosslinking. Actin–DNase I tubes, crystalline actin sheets, and actin filaments at 1 mg/ml were each crosslinked for 10 min by using 0.05% glutaraldehyde. Crosslinking was terminated by the addition of a stoichiometric amount of glycine. Reduced samples in NaDodsO₄ were electrophoresed on either 10% or 8.5% NaDodsO₄/polyacrylamide slab gels (NaDodsO₄/PAGE) according to Laemmli (20).

Preparation of Specimens for Electron Microscopy. Negatively stained specimens were prepared as described (12), with 0.75% uranyl formate (pH 4.25) as the negative stain. Adsorption freeze-drying and unidirectional shadowing with platinum/carbon (Pt/C) were performed according to Smith (21), as modified by Fowler and Aebi (22).

Electron Microscopy. Specimens were examined in a Zeiss EM10C electron microscope at 80 kV accelerating voltage. Micrographs were recorded at 50,000× nominal magnification on Kodak SO-163 electron image film and developed for

Abbreviation: kDa, kilodalton(s).
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4 min in 3× diluted Kodak D-19. Some images were recorded by using minimal dose techniques (12). The magnification was calibrated as described by Wrigley (23) by using negatively stained catalase crystals.

**Image Processing.** Micrographs were screened by optical diffraction to select those containing the most information. Selected areas of the best micrographs were digitized directly with a Photomation P-1700 rotating drum film scanner (Optronics International, Chelmsford, MA) using a scanning raster of 25 μm. Two-dimensional helical filtration and three-dimensional helical reconstruction were performed as described by Smith et al. (24) with a modular interactive micrograph data processing system (25) adapted for use on a PDP11/34 minicomputer. Angular power spectra and rotational averaging of end-on views of rings (e.g., Fig. 1a, arrows) were computed as described by Crowther and Amos (26).

**RESULTS**

**Cocrystallization.** Equimolar mixtures of rabbit skeletal muscle or *Acanthamoeba* actin [43 kilodaltons (kDa)] and bovine pancreatic DNase I (31 kDa) aggregated into ordered tubular arrays (Fig. 1) under precisely the same conditions required for the formation of crystalline actin sheets (12). Briefly, the procedure involves dialysis at 4°C against a low ionic strength buffer to remove excess ATP followed by dialysis at 4°C against a buffer containing 10:1 GdCl3/actin and a variable amount of KCl (12). The formation of actin–DNase I tubes exhibited the same relationship between actin type and ionic strength requirement that was found for the induction of crystalline actin sheets—i.e., 10–25 mM KCl in the case of skeletal muscle actin and 100–150 mM KCl in the case of *Acanthamoeba* actin (12). In addition, the critical concentration of actin—about 0.5 mg/ml—is the same for both actin–DNase I tube and actin sheet formation. When the molar ratio of DNase I to actin was reduced to <1, the number of tubes seen in the electron microscope decreased and, concomitantly, increasing amounts of actin sheets appeared. DNase I alone did not aggregate into ordered structures in the presence or absence of gadolinium at any KCl concentration tried. The same tubular assays were also produced by adding an equimolar amount of DNase I to preformed crystalline actin sheets. This conversion was slow, taking several hours at 20°C, and was absolutely dependent on the presence of DNase I.

**Purification.** Preparations containing tubular arrays were centrifuged at 3,000 rpm for 3 min in a table top centrifuge to remove large, amorphous, or nonspecific aggregation products. The supernatant was then centrifuged for 30 min at 100,000 × g in an Airfuge (Beckman) and the high-speed pellet was resuspended in tube formation buffer. Examination of this material in the electron microscope revealed only tubular arrays against a very clean background (e.g., Fig. 1a).

**Crosslinking.** Fixation of actin–DNase I tubes with glutaraldehyde resulted in the appearance of a crosslinked species having an apparent size of about 88 kDa during NaDodSO4/PAGE on 8.5% polyacrylamide gels. This species comigrated with a crosslinked actin dimer obtained by glutaraldehyde fixation under the same conditions of crystalline actin sheets (Fig. 2b). In contrast, the actin dimer formed by glutaraldehyde fixation of actin filaments (27) under the same conditions migrated with an apparent size of about 163 kDa (Fig. 2a).

**Structural Analysis.** Examination in the electron microscope of negatively stained preparations of actin–DNase I tubes (Fig. la) invariably included both “side” views and “end-on” views (arrows). The side views reveal up to 1 μm long tubes consisting of stacked rings, each of them being 73 Å thick. When viewed end-on, each ring is seen to be composed of seven globular subunits (Fig. 1a, arrows). Comparison of the width of the tubes in both types of view (i.e., 245–255 Å) indicates that these structures are only slightly spread-flattened upon sideview adsorption to the carbon support film. Tubes prepared by adsorption freeze-drying and unidirectional shadowing (21, 22) have a similar overall appearance to those prepared by negative staining (Fig. 1b) but, in addition, demonstrate that the rings in the tubes are stacked on top of each other in a right-handed helix. Also, the end-on views (Fig. 1b, arrow) confirm that the tubes have a hollow core with an average diameter of 120 Å.

Optical diffraction patterns from minimal dose images of negatively stained samples (Fig. 3) usually revealed four orders of meridional reflections, with the first order corresponding to the basic axial repeat (i.e., 73 Å, the thickness of the rings). The second meridional reflection invariably had a larger amplitude than the first, indicating that each 73 Å ring was split into two equal “halves.” The images of the three

![Fig. 1.](image-url) Actin–DNase I tubes prepared for electron microscopy by negative staining (a) and by freeze-drying/metal-shadowing (b). Note the "end-on" views in both types of preparation (arrows, a and b). Note also the right-handed helical striations on the unidirectionally shadowed tube in b. (Bar = 1000 Å.)
FIG. 2. NaDodSO₄/PAGE of purified actin-DNase I tubes (a) and of actin-containing structures that were all crosslinked with glutaraldehyde under identical conditions (b). The four lanes from a 10% gel in a include molecular mass standards (std; in kDa), actin (43 kDa), DNase I (31 kDa), and purified actin-DNase I tubes. The four lanes from an 8.5% gel in b include crosslinked actin sheets, crosslinked purified actin-DNase I tubes, and crosslinked actin filaments. The crosslinked species from the tubes (apparent size, 88 kDa) comigrate with the crosslinked actin sheet dimer (arrow 1), and the crosslinked actin filament dimer migrates with an apparent size of 103 kDa (arrow 2).

best tubes found were digitized as described in Materials and Methods and were used for subsequent digital analysis and processing (24). The apparent 7-fold rotational symmetry of the tubes—as seen in end-on views (Fig. 1a, arrows)—was consistent with the phase relationship of corresponding reflections on either side of the meridian, which pointed to an odd rotational symmetry. These data, together with the layer line altitudes, were used to derive the integer selection rule, \( l = n + 3m \), with \( n = 7n' \) (\( m \) and \( n' \) integers), describing the approximate helical symmetry of the tubes (24).

Using this selection rule, four-helical-repeat long (i.e., 12 axially repeating units) tube stretches (Fig. 4a) were helically filtered (24) (Fig. 4b) prior to separation of the contributions from the top and bottom halves of the tubes by "single-sided" Fourier filtration of the layer lines (24). Such single-sided filtrations (Fig. 4c) reveal that the angularly repeating morphologic unit in the ring is represented by a bilobed stain-excluding region near the center-plane of the ring running obliquely relative to the tube axis (Fig. 4c, arrowhead) with two smaller stain-excluding regions near the top and bottom edges of the ring. The angularly repeating morphologic units in these filtrations display strong 2-fold axes of symmetry, demonstrating that the rings possess a radial dyad axis through their center-plane—a symmetry element that was confirmed upon searching for the corresponding phase relationships.

The helically filtered data (Fig. 4b) of the three best tube stretches were then used to compute three-dimensional helical reconstructions (24). All three reconstructions—one helically repeating unit from one of them is shown in Fig. 5a (left side)—revealed similar structural features whether or not the radial dyad was enforced. When viewed down the tube axis, the reconstructed rings appeared similar to the end-on views seen in negatively stained preparations (Fig. 1a, arrows). This is illustrated in Fig. 5b, where the average over five angularly and translationally aligned, 7-fold symmetrized end-on views (Fig. 5b, left) is compared with the corresponding view (Fig. 5b, right) of the reconstruction.

Side views of the three-dimensional reconstructions of the axially repeating unit (Fig. 5c) are consistent with the major features of the rings seen in single-sided filtrations (Fig. 4c). In particular, the bilobed central stain excluding region (Fig. 4c, arrowhead) was preserved in the three-dimensional model: each of the two equal lobes (marked "A") made contact with respectively the top and bottom sides of the tube (Fig. 5c, arrows). The axial repeat of the tube is 70 Å, and the 7-fold axis is perpendicular to the tube axis.

FIG. 3. One half of an optical diffraction pattern recorded from a minimal dose micrograph of an actin-DNase I tube. The integer helical selection rule (e.g., ref. 24) for this and the other particles used for structure analysis is \( l = n + 3m \), with \( n = 7n' \) (reflecting the 7-fold rotational symmetry of the axially repeating units). In this notation, \( l \) is the layer line number, \( n \) is the Bessel order sampled on layer line \( l \), and \( m \) and \( n' \) are integers (for details, see ref. 24). The corresponding \( l \) and \( n \) numbers for the visible layer lines are indicated as \( l,n \) along the right edge of the diffraction pattern. The 4th-order meridional reflection (12,0) corresponds to a spacing of 18.3 Å. The off-meridional reflections correspond to the helical families running obliquely across the particle (see Fig. 1). [Bar = (50 Å)\(^{-1}\).]
actin and DNase I described here represent a potentially useful form in which to study actin structure and function.

The interaction of actin with DNase I renders the actin subunits nonpolymerizable, possibly by locking the molecule into the "G conformation" of monomeric actin (ref. 29, but see also ref. 6). On the other hand, although actin polymerization is inhibited in the presence of a 6:1 molar excess of gadolinium ions (11), gadolinium-treated actin is apparently not in the G conformation (30); in fact, it has been suggested that such actin might be in a conformation more similar to that in F or filamentous actin (31). Therefore, the gadolinium-induced actin-DNase I tubes described here may provide a link between the molecular models of actin determined from three-dimensional crystals of the actin–DNase I complex, where the actin is presumably in the G conformation, and from gadolinium-induced actin sheets, where the actin is in a distinct, perhaps F-like or intermediate between G and F conformation.

Given the high affinity of the actin–DNase I interaction \( K_a = 5 \times 10^8 \text{ M}^{-1} \) (32), it is likely that the actin–DNase I complex making up the tubes is the same as that which exists in the three-dimensional crystals of the complex, differing only in quaternary structure. However, several lines of evidence suggest that the actin–DNase I tubes are closely related structurally to the crystalline pure actin sheets. (i) Formation of each is induced under identical conditions—i.e., in the presence of a 10:1 molar excess of gadolinium. (ii) The ionic strength required for sheet formation of Acanthamoeba actin is typically five times higher than that required when skeletal muscle actin is used (12), and this same relationship between ionic strength and actin type also applies to the formation of the actin–DNase I tubes. (iii) Preformed actin sheets can be converted into structures morphologically indistinguishable from the actin–DNase I tubes simply by adding an equimolar amount of DNase I. (iv) Crosslinking of each of the two structures with glutaraldehyde results in the formation of crosslinked species that comigrate during NaDodSO4/PAGE with an apparent size of 88 kDa (Fig. 2b). Because the soluble actin–DNase I complex is resistant to crosslinking by glutaraldehyde under the same conditions (data not shown), it is likely that both of these crosslinked species represent the same type of actin dimer, which however is different from the actin dimer (migrating with an apparent size of 103 kDa) obtained when filaments are crosslinked under the same conditions (Fig. 2b). (v) The repeating units of both the actin–DNase I tubes and the crystalline actin sheets contain a dyad axis of symmetry. Each ring in the actin–DNase I tubes contains a radial dyad axis centered on a bilobed stain-excluding region (Fig. 5a, left), whereas the unit cell of the actin basic sheet (9, 11, 12) contains two actin molecules related by a 2-fold axis perpendicular to the sheet plane (Fig. 5a, right).

Taken together with considerations relating to the dimensions and size of the actin dimer, these data are consistent with the bilobed stain-excluding region around the centerplane of the ring reconstruction representing an actin dimer (Fig. 5c, indicated schematically by a dotted dimer), which is probably identical to that found in the crystalline actin sheets. An immediate consequence of this choice is the identification of the smaller, peripheral stain-excluding domains (Fig. 5c, marked D) as representing mainly DNase I. Therefore, the radial dyad axis in the center-plane of each ring relates actin–DNase I complexes in the upper half of the ring to those in the lower half. In this arrangement, the intermolecular contacts between the upper and lower half-rings are brought about via seven actin dimers. A further consequence is that intermolecular contacts between adjacent rings in the tube exclusively involve interactions between DNase I molecules.

Identification of the radial dyad axis in the actin–DNase I
tube with the dyad axis in the unit cell of the actin basic sheet is crucial in the assignment of the actin and DNase I moieties given in Fig. 5c. As to the identification of the actin–DNase I complex in the reconstruction, we are left with two choices because each actin subunit in our model makes contacts with two DNase I molecules (Fig. 5c). One way to try and resolve this ambiguity will be to fit the actin–DNase I complex as it has been determined by x-ray diffraction analysis (7) into the actin–DNase I tube model presented here.

As to the relative alignment of the actin molecule in the tube and in the sheet, the exact angular orientation and the polarity of the actin sheet dimer relative to the dyad axis in the tube have yet to be established. Unfortunately, beyond the presence of the dyad axis, the shape of the actin dimer region in the tube reconstruction and that of the actin dimer in the actin sheet reconstruction do not look all that similar (Fig. 5a). This may be due to the limited order of the tubes as compared to that of the sheets with resulting discrepancies in the respective reconstructions. Alternatively, the apparent differences in the dimer shape may be interpreted in terms of possible differences in the conformation of the actin molecule in the two structures—i.e., in the presence and absence of DNase I (see above).

Nevertheless, a more detailed analysis of the structure of the actin–DNase I tubes may yet provide the means by which the x-ray map of the actin–DNase I complex (7) and the molecular model determined using negatively stained crystalline actin sheets (9) can be aligned with respect to each other and ultimately within three-dimensional reconstructions of negatively stained actin filaments.

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