Hypothesis

What is the diameter of the actin filament?

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Abstract The limits of the most recent models of the actin filament are discussed. These model are generated without taking into account the effect of protein osmotic pressure and, in general, of the solvent conditions. As a result they do not provide a bona fide representation of the actin filament in vivo. A new 'fluttering wing' model is proposed which predicts that orientation of the monomers, intermonomer contacts and diameter of the actin filament are sensitive to protein osmotic pressure and to interaction with regulatory proteins.

1. Introduction

In the last few years many efforts have been devoted to solving the structure of the actin filament — a tremendous task due to its huge dimension. A few models were presented which can be classified as follows: (a) models generated from optical diffraction of actin paracrystals [1–3]; (b) Heidelberg model generated from X-ray fiber diffraction pattern of an oriented gel of F-actin [4–7]; (c) ribbon-to-helix transition model generated from X-ray fiber diffraction of crystalline profilin: β-actin [8–11]; and (d) Hegelman-Padron model generated from X-ray diffraction of live relaxed muscle [12].

In addition to these models, all of which depict the actin filament as a rigid structure, we propose (e) the 'fluttering wing' model which predicts a variable diameter as well as a variable orientation of the monomers of the actin filament [13–15].

We wish to compare the 'fluttering wing' to the 'rigid' models and to stress the major pitfals of the latter.

2. Models for a rigid-structure actin filament

2.1. Models generated from optical diffraction of actin paracrystals

The earliest models are generated from optical diffraction of actin paracrystals. The contours of the monomer masses are delined and, in the regulated actin filament, the electron density contribution of tropomyosin is resolved. The diameter of the actin filament is predicted to range between 8 [1] and 7 nm [2]. Often these models are used to provide the framework for higher resolution reconstructions.

2.2. Heidelberg model

This model was generated by comparing the X-ray fiber diffraction pattern of an oriented gel of F-actin [16] with the X-ray diffraction pattern of the actin monomer in the actin–DNAse I complex [4]. In a first trial, the four domains of G-actin were considered independently as rigid bodies [5]; the model was further refined by the 'directed mutation algorithm' method [6]. Finally, the slow normal modes of G-actin were used as structural parameters to refine F-actin [7]. In all the procedures the same set of X-ray diffraction data from oriented gel of F-actin was used. As a result of these studies, the location and nature of a number of possible intermolecular bonds between adjacent actin monomers were defined. A diameter of 9.5 nm was assigned to F-actin [6]. These authors clearly warn that the assignments are tentative: "the significance of details of the interactions is not high, since they mostly result from changes induced by heating and cooling in X-PLOR and are not linked to experimental data" [6].

2.3. Ribbon-to-helix transition model

The 2.55 Å resolution of the structure of crystalline profilin–β-actin allowed Schutt and colleagues [8–11] to present a completely different model of polymeric actin. The authors formulated the hypothesis that actin may form a ribbon (diameter: 9.5 nm) capable of undergoing a ribbon-to-helix transition into the actin filament [11]. They further proposed [8,9,11] that the profilin–actin contact and the two-start helical contact in F-actin are mutually exclusive so that addition of profilin to F-actin converts the actin filament into a ribbon where only the one-start helical contact is conserved. The removal of profilin restores the two-start helical contact and allows the formation of F-actin. The ribbon-to-helix transition would take place through a 13° twist and 8.3 Å shortening per monomer [10]. Actually, this model is incompatible with the F-actin model proposed by Lorenz et al. [6] since it does not use the same one-start helical contacts [17].

2.4. Hegelman-Padron model

This model was generated by using the integrated intensity of the 5.9 nm layer line from the X-ray diffraction pattern of live relaxed frog sartorius muscle and the phases determined by electron microscopy of negatively stained, isolated actin filaments [12]. The conclusion of these studies was that in vivo the actin filament displays a diameter of 10 nm.

3. Fluttering wing model

The idea that the diameter of the actin filament may change with the experimental conditions stems directly from the literature. Using the thin sectioning technique, the diameter of the actin filament in muscle was found to vary from 5 [18] to 7 nm [19]. Urged on by this discrepancy we studied the effect of protein osmotic pressure (protein concentration) on the volume of the hydrated actin filament and showed that the diameter of the actin filament decreases significantly with an increase in protein osmotic pressure [13–15].

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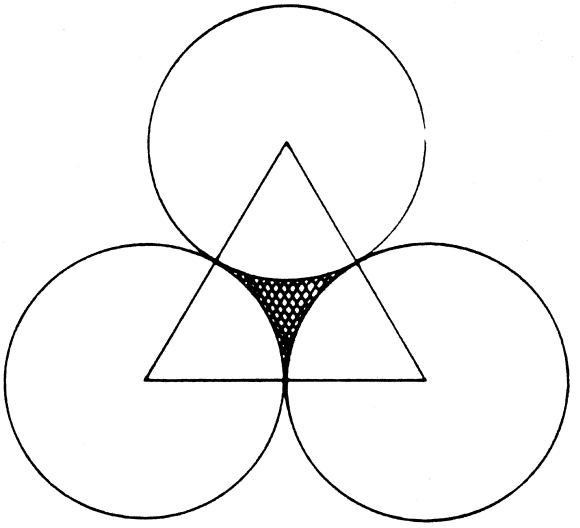

Fig. 1. Cross-section of hexagonally packed actin filament: the elementary unit. The side of the equilateral triangle is the interfilament distance (d).

This could be done because in vitro, at 1.8×10^5 dynes/cm^2 (the protein osmotic pressure in frog muscle [20]), actin filaments pack hexagonally into bundles of filaments (Fig. 1). As a consequence, protein molality (m) and interfilament distance (d) correlate according to the equation:

$$d=\left(\frac{[(vxM_rxm)+1005]x4}{mxNx2.73x10^{-7}x2\sqrt{3}}\right)^{1/2}\ \text{cm}$$

where v is the partial specific volume of F-actin (0.718 ml/g) [21], M_r is the monomeric mass of F-actin or the equivalent mass of regulated F-actin; N is the number of Avogadro; 2.73×10^{-7} cm is centimeters of filament per actin monomer [31]; and 1005 is the water + salt volumes in cm^3 [13,15].

Due to the hexagonal packing of actin filaments (Fig. 1), simple geometrical considerations [15] allow partitioning of the total volume of the solution:

$$V=\text{moles of actin}\times N\times2.73\times10^{-7}\times2\sqrt{3}\,d^2/4\ \text{cm}^3$$

(where $2\sqrt{3}\,d^2/4$ is the area of the cross-section of the elementary unit of hexagonally packed actin filaments) into the volume fraction of interfilament water:

$$V\times\left(1-\frac{\pi}{2\sqrt{3}}\right)=0.093\times V$$

and the volume fraction of the hydrated actin filament:

$$V\left(\frac{\pi}{2\sqrt{3}}\right)=0.907\times V.$$

Once allowance has been made for the volume occupied by the protein, it turns out that *intrafilament water* volume cannot be less than 45% of the total volume of the solution. It is thus clear that the water fluxes, concomitant with the changes of protein osmotic pressure, are mostly supported by *intrafilament* water. The efflux or influx of this structural water certainly influences the architecture of the filament, i.e. the orientation of the monomers and intermonomer contacts.

Our model is formulated on the assumption that actin filaments do not interpenetrate while being submitted to an osmotic stress and that decrease in the volume of the solution is concomitant with the decrease of the volume of the hydrated actin filaments. We assume that, beyond a given pressure, change in the volume of the solution is accompanied by change in the shape of the filaments. This change in shape is modeled by the decrease of the angle formed between the long axis of the monomer and the pointed end of the filament axis [14]. Other representations of the change in shape could also be used without affecting significantly their relationship with the changes in volume.

The contrary assumption that filaments do indeed interpenetrate does not require that the diameter of the hydrated actin filaments changes with protein osmotic pressure.

We know from electron microscope observations that, even at the highest protein osmotic pressure employed in our experiments, the filamentous structure of F-actin is conserved. Furthermore, since the filaments are helicoidal, we expect that significant interpenetration should be accompanied by supercoiling of the filaments. This is certainly not the case; actin filaments run side by side in the bundles [22] — a feature incompatible with supercoiling of the filaments. Nevertheless, we admit to being unable to exclude, on this basis, a partial interpenetration of the filaments.

The real proof that interpenetration of the filaments is not a significant phenomenon actually resides on the physically untenable contention that osmotic stress is not accompanied by a change in the shape of the filaments, i.e. by bending of the monomer with respect to the filament axis. If this were true, stiffness of the monomer in the filaments should tend to infinity — an assumption rejected both by good sense and experimentation. The elastic modulus by stretching for skeletal muscle fibres has finite values [23,24]. Stretching in skeletal muscle involves, beside the sign, the same solicitation of the crossbridge as the bending of the monomer in F-actin in vitro. Thus bending of the monomer and stretching of the fibre are identical. It follows that the monomer, as well as the crossbridge, can be deformed elastically either by a mechanical [23,24] or an osmotic force [14,25,26]. As a result of this elastic deformation, extensive reorientation of the monomers in the filament and, possibly, extensive rearrangement of the intermonomer contacts must occur.

4. Solvent and molecular modelling

In the modelling of the actin filament influence of the solvent is usually ignored. As an example, the Heidelberg model is generated by comparing the X-ray fiber diffraction pattern of an oriented gel of F-actin at the protein osmotic pressure of $\sim10^4$ dynes/cm^2 [16] with the X-ray diffraction pattern of the actin monomer in the actin–DNAse I complex, crystallized from 8–10% polyethyleneglycol at the protein osmotic pressure of $\sim10^6$ dynes/cm^2 [4]. As a matter of fact, the actin monomer, equilibrated to a protein osmotic pressure of 10^6 dynes/cm^2, when exposed to a protein osmotic pressure of 10^4 dynes/cm^2, picks up a huge amount of protein solvation water (tens of liters per mole of monomer). Thus it is difficult to believe that the position of the 'large' (domains 3 and 4) with respect to the 'small' domain (domains 1 and 2) and, consequently, the overall shape of the monomer, are not altered by a shift from the higher to lower protein osmotic pressure. Furthermore, since hydrogen-bonding, electrostatic and hy-

drophobic forces are totally dependent on the state of the water solvent, structural changes, intrinsic to the four small domains of the monomer, are also expected to occur. Some structural changes are indeed noticed between 'monomeric' (in fact the actin–DNAse I complex) and F-actin: 15° twisting for subdomain 2 and 2–3° movement for the other domains [5]. Unfortunately the contribution of DNAse I and protein osmotic pressure to these changes cannot be resolved.

5. In vivo versus Heidelberg model actin filament

From the considerations made above, it is expected that the in vivo actin filament is significantly smaller than that calculated using the Heidelberg model. In fact the latter is generated at a protein osmotic pressure significantly smaller than that occurring in vivo: 1.8×10^5 dynes/cm^2 in frog muscle [20]. Assuming a cylindrical shape for the hydrated actin filament (a very crude assumption but good enough for the point I wish to make), the volume of a gmole (as actin monomer) of the Heidelberg filament (diameter: $\approx$9.5 nm) is 116.6 l at the protein osmotic pressure of 10^4 dynes/cm^2 and decreases to 82.7 l (diameter: $\approx$8 nm) at the protein osmotic pressure of 1.8×10^5 dynes/cm^2 [13,15].

Thus the Heidelberg model may correctly represent the pure actin filament at the macromolecular osmotic pressure of 10^4 dynes/cm^2 but it does not represent a pure actin filament at the protein osmotic pressure in vivo. Actually, that something is wrong with the present modeling of the actin filament is also recognized by others. Bremer and al. [3], after a number of attempts to provide a three-dimensional reconstruction of the F-actin filament, observe "only assuming a significantly lower protein density of approximately 0.5 to 0.55 Da/A^3 for the F-actin subunit can simultaneously yield the correct filament diameter and the correct molecular volume".

6. Rearrangement of the intermonomer contacts in F-actin

In the 'rigid body' model of the actin filament it is proposed that the strongest interaction is brought about by formation of an extensive hydrophobic core between neighboring molecules along the two-start helix [5]. Hydrophobic packing is relatively aspecific; thus there is no reason why the actin filament, submitted to protein osmotic stress, could not stand an extensive reorientation of the monomers, provided that the overall area of the interacting hydrophobic surfaces is not largely decreased. Sliding of the contact surfaces of the actin monomers does not require a simultaneous breaking of all the monomer–monomer contacts (ΔG 30–40 Kcal/mol). It may occur as a bond breaking–bond forming process, the overall required energy thus being very small.

This reasoning seems to contradict the "principle of conserved contacts with variable linkages between multi-domained subunits". The 'principle' is essentially based on an oversimplified interpretation of the crystallographic work of Robinson and Harrison [27] on the expanded form of the tomato bushy stunt virus. It is true that the model for expansion assumes that "the virus retains its icosahedral symmetry and that the internal tertiary structure of each domain is conserved". It is also true, however, that "about six residues immediately N-terminal to the A and B position, S-domains of the compact structure become ordered in the expanded state, extending the first strand of each β-sheet and forming hydrogen bonds with residues of the opposite subunit". This means that the internal tertiary structure of the domains is *not* conserved since, after expansion, new hydrogen bonds are formed and a strand of a β-sheet is elongated. This is exactly what we propose to occur when F-actin is subjected to a protein osmotic stress.

7. Pitfalls of the Egelman–Padron model

Discussion of the model of Egelman and Padron [12] might present some ambiguities. We indicate that, at the putative *physiological* protein osmotic pressure, the diameters of pure F-actin and of regulated F-actin, either in 2 mM EGTA or in 0.2 mM $CaCl_2$, are 8.3, 13.3 and 12.2 nm, respectively [15]. We are unable to trace the contour of the actin monomers in the filament; thus we cannot exclude that regulated F-actins display larger diameters because of the outward projections of tropomyosin and troponins.

Egelman and Padron [12] maintain that, *in relaxed living muscle*, the diameter of the actin filament (regulated actin filament at very low Ca^{2+} concentration) is 10 nm. Since they analyze the 5.9 nm layer line, this indicates that the contour of the monomer masses extends up to a radius of $\sim$5 nm.

These combined informations could mean that, at fairly the same protein osmotic pressure, the contour of the monomer masses extends up to the radii of $\sim$4 nm in pure actin filaments and of $\sim$5 nm in regulated actin filaments. The indication could support our model which predicts that the orientation of actin monomers is influenced by interaction with ancillary proteins.

Unfortunately the work of Egelman and Padron [12] raises some reservations. These authors present three lines of evidence.

(a) Hanson et al. [28] observed a sixth layer line meridional reflection in the X-ray diffraction pattern of pure actin gel (low protein osmotic pressure) that disappeared when the mean interfilament spacing was slightly greater than 10 nm. In the opinion of Egelman and Padron [12], the reflection arises when actin filaments are in direct contact; thus the diameter of the filament must be 10 nm. We do not question this interpretation. The proposal of Egelman and Padron [12] confirms our view that, at low protein osmotic pressure, the actin filament displays a large diameter.

(b) The giration radius of the actin filament is the same as that of an homogeneous cylinder having a radius 7.1 nm. Since F-actin is not a compact cylinder, but rather has a deep helical groove along the 5.9 nm helix, the maximum diameter of the actin filament must be significantly larger than 7.1 nm. The radius of giration [29] was determined on actin filaments subjected to a very low protein osmotic pressure (actin concentration: 6–28 mg/ml). Thus Egelman and Padron [12] only show that the diameter of the actin filament, at low protein osmotic pressure, is larger than 7.1 nm. We are in agreement.

(c) Egelman and Padron [12] claim that, in live muscle, the actin filament diameter is 10 nm. The model is generated by using the integrated intensity of the 5.9 nm layer line from the X-ray diffraction pattern of live relaxed frog sartorius muscle and the phases determined by electron microscopy of negatively stained, isolated actin filaments. This procedure is correct only if the two objects, the actin filament in vitro and the

thin filament in muscle, are identical. If the two objects are different, the phases determined for one object cannot be used to build the model of the second object. Thus, Egelman and Padron [12], by employing the phases determined from isolated actin filaments (at low protein osmotic pressure), imply that the isolated actin filament and the thin filament in muscle are identical. Why then construct a model of the filament in muscle?

According to Egelman and Padron [12], "the model data match the X-ray data well". Good matching, however, could be apparent. The shape of the monomer in the model (two overlapping spheres) is quite different from the shape of the monomer in F-actin and it is known [30] that subunit shape contributes to determine layer-line strength.

We think that these data are inadequate to either support or deny our hypothesis.

8. Conclusion

A reliable modeling of the actin filament requires that the influence of the solvent is carefully taken into consideration. The requirement becomes more stringent as higher is the resolution of the model worked out.

The fact that the orientation of the actin monomer in the Heidelberg model is consistent with a number of independent biochemical and biophysical measurements is not surprising [5,6]. All measurements were performed under solvent conditions similar to those employed to generate the model (i.e. at low protein osmotic pressure) and very different from those prevailing in vivo.

If we wish to unravel the mechanism of muscle contraction, we need models generated under near in vivo conditions.

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