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Geometrical Analysis of F-actin Helix

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Geometrical analyses are performed on the relationship between pitch length and rotational angle per monomer for F-actin helix, which is constructed by spherical monomeric units. The pitch length of 700 Å and the number of monomers per turn, 26, observed in the electron micrograph by Hanson and Lowy, give rise to the diameter of 55 Å by using the relation that each sphere must contact with neighboring four spheres, two in the same strand and the other two in the different strand, and the interaction sites on the monomer are the same for every monomer. A slight deviation of the sites in position, however, results in a drastic change of helical dimension, *e.g.*, movement of the sites by 0.3 Å yields the change in pitch length as much as 30 Å. A plausible helical dimension of F-actin helix is discussed considering the interaction with other muscle proteins, tropomyosin, troponin, and myosin.

INTRODUCTION

Proteins such as muscle protein F-actin, bacterial flagella and microtubule are linear assembly of unit monomers,[1,2] each of which is a spherical protein of molecular weight about 50,000. Morphology of these polymers has been found by means of electron microscopy that monomers are connected into a long fiber by non-covalent bonds, and the fibers in some case associate each other side-by-side to form a relatively stiff fibril.

F-actin, which is one of the well-investigated proteins, is a typical one constituted of two stranded double helix of linear polymers with a pitch of 700 Å and 13 monomers per one turn for a single stranded fiber according to the model proposed by Hanson and Lowy[1] based on the electron micrograph. Actin monomer, therefore, has two pairs of interaction sites, one connecting monomers to form a strand of helix and the other associating two strands. In addition, all the monomer molecules are identical proteins,[3] so that the binding sites must locate at the same positions on the surface of the molecule.

If we simplify the system by the approximation that spherical molecules constitute a double helix of a given geometry, geometrical analysis may be performed on the accuracy of the location of the binding sites on the surface to build up the double helix and effects of the change in the sites on the helical geometry.

CALCULATION OF GEOMETRY

When two necklace-like polymers, each being made by spherical monomeric units with a radius R, take a double helical form of a given pitch length p and rotational angle θ per one monomer along the helical axis, we have following equations,

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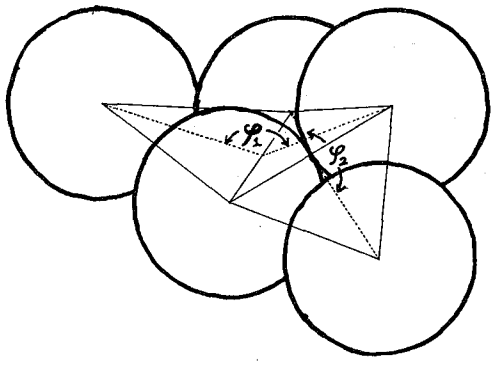

Fig. 1. A double helix may be characterized by a dihedral angle φ between successive regular triangles, apexes of which are centers of spheres.

$$(2\,r\sin(\theta/2))^2 + p^2 = 4\,R^2 \tag{1}$$

$$(2\,r\sin(\theta_0/2))^2 + p_0 = 4\,R^2 \tag{2}$$

$$(2\,r\sin((\theta-\theta_0)/2)^2 + (p-p_0)^2 = 4\,R^2, \tag{3}$$

where p_0 and θ_0 are relative pitch length and rotational angle of one polymer H_1 to the other H_2, and r is the radius of each helix with respect to the center of the sphere. These equations are derived from the condition that every sphere must contact with neighboring four spheres as shown in Fig. 1. The coordinates of i'th monomer in the helices, H_1 and H_2, may be expressed by using a cylindrical coordinate system as,

$$\begin{aligned} x_i &= r\cos(i\theta) & x'_i &= r\cos(i\theta+\theta_0) \\ y_i &= r\sin(i\theta) & y'_i &= r\sin(i\theta+\theta_0) \\ z_i &= ip & z'_i &= ip+p_0. \end{aligned} \tag{4}$$

The relative position of the second helix H_2 to H_1 may be determined by the solution of equations (1), (2), and (3) for a given value of R, p, and θ. There are two solutions of the equations, one in general and the other in special as described below.

Since the double helix may be characterized by the successive connection of regular triangles formed by centers of constituent spheres whose sides are equal to $2R$, the helix may be described in terms of dihedral angles φ_1 and φ_2 between two regular triangles out of three neighboring ones (Fig. 1). These angles are related to p, θ, p_0, and θ_0 by the equations,

$$3R^2\cos\varphi_1 = (1/4+\cos(\theta+\theta_0)-\cos((\theta+\theta_0)/2)\cos((\theta-\theta_0)/2))r^2 - 1/4(p+p_0)^2 \tag{5}$$

$$3R^2\cos\varphi_2 = (1/4+\cos(2\theta-\theta_0)-\cos((\theta-\theta_0)/2)\cos(\theta_0/2))r^2 - 1/4(2p-p_0)^2 \tag{6}$$

When φ_1 and φ_2 are different, the position of the second helix is determined by φ_1 and φ_2 for a given p and θ, that is, translation of the first helix by p_0 along the axis and rotation by θ_0 gives the position of the second helix, forming a double helix. This is the general solution of Eqs. (1), (2), and (3). Another solution of the equations, which is valid within limited values of p and θ, is obtained for $\varphi_1=\varphi_2$, or

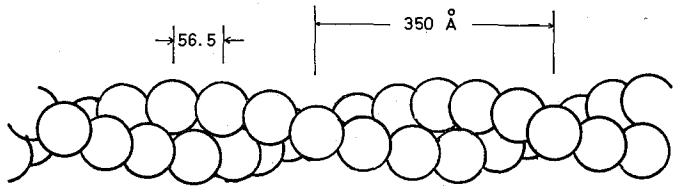

Fig. 2. A model for F-actin double helix, and the dimension given is that of Hanson and Lowy.

$$\theta_0 = \theta/2+\pi, \qquad p_0 = 1/2p. \tag{6}$$

We are interested in the double helix of this solution because the helix for this solution can be made from identical spheres having the same binding sites (Fig. 2), whereas the first solution does not give the same interaction sites as clearly seen by non-identity of φ_1 and φ_2.

Since θ_0 and p_0 are given in Eq. (6), Eqs. (1), (2), and (3) reduce to

$$\begin{aligned}&(2r\sin(\theta/2))^2 + p^2 = 4R^2\\&(2r\cos(\theta/4))^2 + p^2/4 = 4R^2.\end{aligned} \tag{8}$$

Then we have simple relations as follows,

$$\begin{aligned}p &= 2R(1 - 3\tan^2(\theta/4))^{1/2}\\r &= (3^{1/2}/2)\, R\sec^2(\theta/4)\end{aligned} \tag{9}$$

Thus, the helix is specified for a fixed radius of monomer sphere once one of the variables, p or θ is given. For Hanson and Lowy model of F-actin,[1] 13 monomer units per one turn and a length 700 Å of the helix, $2R$ is calculated to be 55.08 Å and $r=$ 24.2 Å. When m subunits are contained in one turn of a helix, the spacing of one turn P and rotational angle per one subunit θ are written as $P=mp$ and $\theta=2\pi/m$. Figure 3 demonstrates the dependence of P on m as a function of $2R$ obtained by equation (9). This relation may be utilized for determination of R from the geometry observed in electron micrographs of F-actin. The dihedral angle, φ, between two triangles is represented as a function of m in Fig. 3. As seen in the figure, a slight deviation of the angle gives rise to a large change in helical parameters P and m or θ. Such a change is expected to occur by attachment of ligands or proteins, *i.g.*, tropomyosin and troponin.

INTERACTION SITES ON MONOMERIC UNITS

The double helix described previously is constructed by the monomer spheres which have the same set of four interaction sites. The coordinates of these sites are,

$$\begin{aligned}a_1 &= (-r\sin^2(\theta/2), &&-r/2\sin\theta, &&-p/2)\\a_2 &= (-r\cos^2(\theta/4), &&r/2\sin(\theta/2), &&-p/4)\\a_3 &= (-r\cos^2(\theta/4), &&-r/2\sin(\theta/2), &&p/4)\\a_4 &= (-r\sin^2(\theta/2), &&r/2\sin\theta, &&p/2)\end{aligned} \tag{10}$$

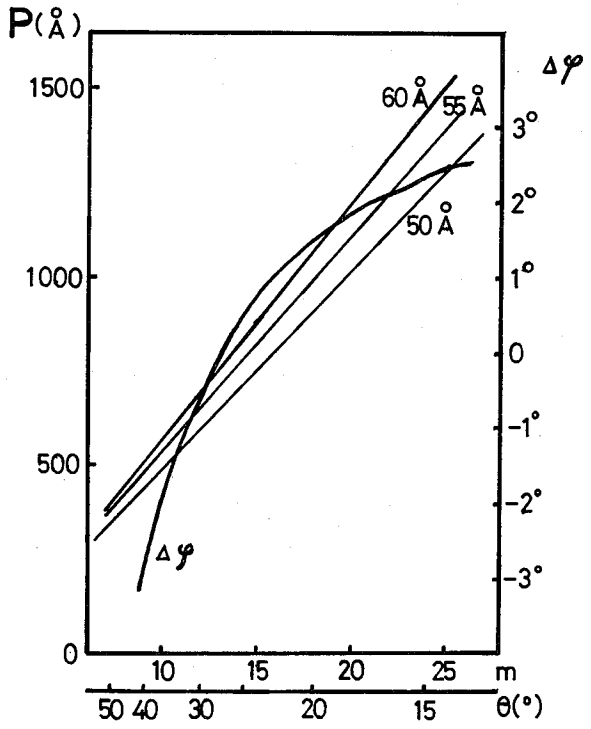

Fig. 3. Helical spacing $P=m\,p$ is plotted against the number of subunits of one strand per turn, m, or rotational angle per monomer, θ, as a function of the diameter of spheres. The deviation of the dihedral angle φ from $\varphi_0=139.7°$ ($m=13$), $\Delta\varphi$, is plotted against m.

expressed by the cylindrical coordinate system of the origin at the center of the sphere and z axis parallel to the helical axis. Since r and p are given by Eq. (9), the coordinates, a_i, are determined as a function of θ and R. These coordinates may be transformed to a polar coordinate, R, Θ, and Φ, which are more convenient for the expression of a_i. For instance, the values of Θ and Φ for $m=13$ are listed in Table I and shown in Fig. 4.

These sites are not equivalent in general but has specificity for the interaction: site 1 interacts with site 4 to make one strand of the double helix, and site 2 interacts with site 3 to associate two strands together. According to the geometry, angles between site 1 and 2, 2 and 3, and 3 and 4 are 60°. As demonstrated in Fig. 4, these sites are almost in a plane for Hanson and Lowy model. Experimental results on polymerization of actin[3] indicate that ADP and divalent cation are involved in the interactions between these sites.

Table I. Θ and Φ for $m=13$

site	1	2	3	4
Θ(°)	257.8	209.3	150.3	102.2
Φ(°)	256.2	187.0	173.0	103.8

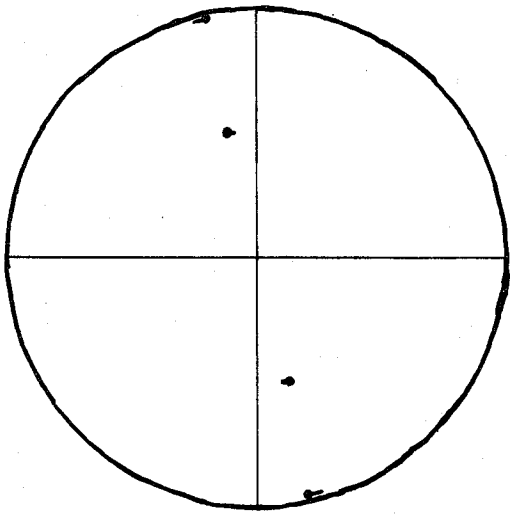

Fig. 4. A view of interaction sites on the action monomer for $m=13$. Solid bars indicate displacements of the sites when m changes from 13 to 18.

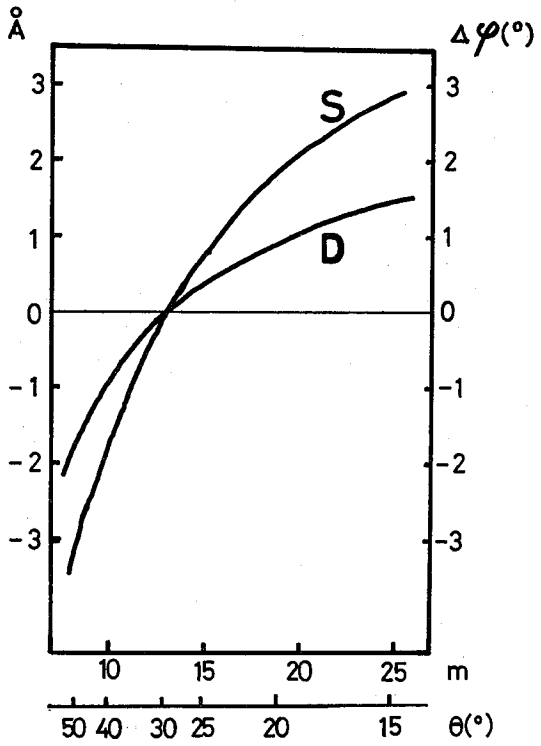

Fig. 5. Displacements of interaction sites responsible for the same strands (S) and for the different strands (D) are expressed as a function of m or θ.

As analysed before, a slight movement of these sites gives rise to the change in helical geometry through θ. Figure 5 demonstrates the displacement of the interaction sites within the same strand (S) and between different strand (D) as a function of m for R=55.08 Å. The result indicates that the change in m by one causes the movement of the interaction sites as small as 0.3–0.4 Å. In other words, a small conformational change of the monomers induced by the ligands is sufficient to produce a large increase or decrease in helical spacing.

DISCUSSION

The experimental geometry of F-actin obtained from the electron microscopic data is a double helix of half pitch length of 350 Å composed of 13 monomers with a diameter of 55 Å, which satisfy the geometrical condition as shown in Fig. 3. According to the model of thin filament proposed by Ebashi,[4] tropomyosin molecules of 400 Å in length are present along the grooves of two actin strands, and troponin, which is a regulatory protein, binds on every tropomyosin molecule. Both X-ray diffraction,[5] and electron microscopic[6] studies indicate that the periodicity of troponin or tropomyosin (because one troponin binds to one tropomyosin) is 380–385 Å along the axis.

As shown in Fig. 3, the actin helix can be specified once one of the helical parameters is given at a fixed diameter of a monomer molecule (here we shall use a value of 55 Å). Therefore, we can calculate the length of a fiber which forms a helix of the same pitch but a different radius r. Figure 6 shows curves of a molecular length L versus r as a function of the helical pitch. When a tropomyosin molecule winds along an actin double helix in half a turn, the pitch of the helix should be 760 Å and its radius where tropomyosin exists will be 38 Å. Similarily we can also compute the spacing when 400 Å molecules lie along the grooves of actin strands at various radius; 385 Å at r=32 Å, 380 Å at r=38 Å and 375 Å at r=44 Å almost independent of the size of actin helix. Therefore, tropomyosin molecules exist presumably along the actin helix at a radius of 32 to 38 Å, as postulated by the X-ray diffraction and electron micrographic studies.[7]

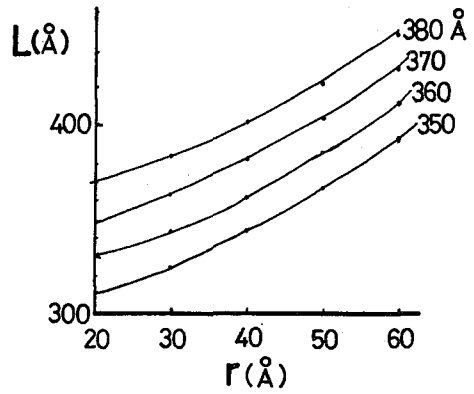

Fig. 6. The length of helical fiber around the double helix versus the radius is shown as a function of half spacing of the double helix.

It may be worth mentioning that the increase in r results in reduction of the spacing along the axis, so that some change in spacing of tropomyosin must be detected if the position of tropomyosin changes from a relaxed state to a contracted state. However, no significant change has been observed experimentally for the spacing of 380 Å, suggesting that the radius of tropomyosin helix remains constant.

Another geometrical consideration about the actin helix may be performed on the arrangement and interaction with respect to myosin filament or thick filament. Since thin and thick filaments are packed in hexagonal lattice perpendicular to the filament axes, and actin and myosin are conceivable to interact at the same sites, spacing due to myosin found by electron microscope and X-ray diffraction,[8] 430 Å, must be elucidated in terms of geometry and interaction with the actin filament. If we take the experimental value of 700 Å per 26 monomers, the interaction occurs at the position about 3/5 turn of the actin helix, which is far from the six fold symmetry. Since a slight change in m makes the drastic change in the pitch length of actin helix, we assume that m changes 13 to 12, then pitch length reduces to 644 Å. For this helix, the interaction occurs at the position of 2/3 of the helix, which gives just three fold screw symmetry favorable for the interaction of actin and myosin. We have the experimental data for the periodicity of 380 Å for troponin, 51 and 59 Å for actin, and 430 Å for myosin, so that there is no contradiction about this postulate. There is, however, no experimental data to show the evidence of change in m when F-actin interacts with myosin fiber. Since this postulate satisfies the interaction behavior and the reasonable arrangement of thin and thick filaments on the geometrical stand point, it is presumed that the actin helix interacting with thick filament might have different spacing than F-actin fiber in vitro.

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