Small-angle X-ray scattering of the actomyosin solution

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Geometric parameters of actomyosin in Weber—Edsal solution at the concentration of 1 mg/ml, diluted 1 : 100 by physiological solution were determined by the method of small-angle X-ray scattering. It was found that the solution of actomyosin consists of two fractions with fibre cross-section radii $r_1 = 8.55 \pm 0.09$ nm and $r_2 = 13.2 \pm 0.2$ nm.

Методом рассеяния рентгеновского излучения на малые углы были определены геометрические параметры актомиозина в растворе Вебера–Эдсала с концентрацией 1 мг/мл, разбавленном физиологическим раствором в соотношении 1 : 100. Было обнаружено, что раствор актомиозина содержал две фракции с радиусом сечения волокон $r_1 = 8{,}55 \pm 0{,}09$ нм и $r_2 = 13{,}2 \pm 0{,}2$ нм.

The pioneer studies of *Engelhardt* and *Lyubimova* [1], *Szent-Györgyi* [2] and some of his coworkers evoked intensive investigations of properties of contractile muscle proteins and of the terminal mechanism of muscle contraction. The investigation of the ultrastructure of muscle fibres by electron microscopy [3—5] and of small-angle scattering [6, 7] along with biochemical and biophysical characteristics considerably contributed to our knowledge in this field. Geometric parameters of macromolecules in solutions can be studied by the method of small-angle X-ray scattering. Therefore we examined in a preliminary experiment the applicability of this method to the investigation of geometric properties of actomyosin aggregates, as well as of the aggregation ability of actin and myosin.

Experimental

Actomyosin was isolated by the method of *Szent-Györgyi* [8] which was modified for our special purposes. A dog heart muscle was homogenized with five volumes of Weber—Edsal solution (0.6 M-KCl, 0.03 M-$NaHCO_3$, 0.01 M-Na_2CO_3). After 5 min of standing the

homogenate was centrifuged with grounded glass and the supernatant was diluted by ten volumes of distilled water. After flocculation the suspension was centrifuged and the gel of actomyosin was dissolved in three volumes of Weber—Edsal solution. The procedure was then repeated four times in order to exclude the occurrence of free actin and myosin. The period of the actomyosin extraction from the muscle has been shortened in order to obtain an extract relatively lacking in the actin content in comparison with physiological conditions. The purpose of the experiment was to prevent the formation of large spherical aggregates of the actomyosin. The procedure gave the actomyosin concentration of some 1 mg/ml solution of pH 7.2. Before the measurement the solution was diluted 1 : 100 by physiological solution.

The small-angle scattering measurement was made with a sample of actomyosin solution placed in Mark capillary 1 mm in diameter, using CuK_α irradiation filtered by filters of *Ross* [9]. The effect of a collimation on the scattering curve was eliminated "by hand" according to [10].

Results

Actomyosin belongs to fibrillar proteins. Therefore we used for the calculation of geometric parameters of the molecule the modified method of *Kratky* and *Porod* [11, 12]. The intensity of scattering of needle-shaped particles of "infinite" length is given by

$$\ln (I\Theta) = \ln (I\Theta)_0 - \frac{16\pi^2}{2\lambda^2} R_q^2 \Theta^2 \qquad (1)$$

where Θ is Bragg's scattering angle and R_q is the radius of inertia of the scattering object. If the fibre cross-section is considered, in the first approximation, to be circular its radius is expressed by

$$r = \sqrt{2} R_q \qquad (2)$$

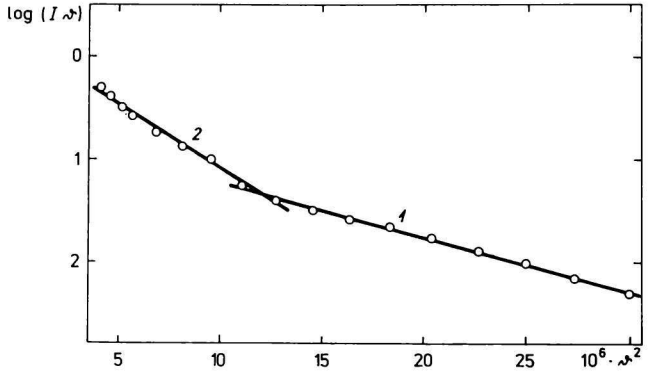

Fig. 1. Scattering curve of actomyosin.

The scattering curve was measured in the angle range from 2.02×10^{-2} rad to 5.47×10^{-3} rad. The intensity values relieved of collimation effect were plotted in a diagram of $\log (I\Theta) = f(\Theta)^2$, shown in Fig. 1. From the course of the curve it is obvious that the measured actomyosin solution consists of two fractions. The radii of inertia calculated according to eqn (*1*) using the slopes of both straight lines were found to be $R_{q1} = 6.04 \pm 9.6$ nm and $R_{q2} = 9.34 \pm 0.15$ nm. The radii of fibre cross-section calculated according to eqn (*2*) were found to be equal to $r_1 = 8.55 \pm 0.09$ nm and $r_2 = 13.2 \pm 0.2$ nm.

Discussion

Myosin belongs to fibrillar proteins of α-keratin type. The fibre length equals about 160 nm, relative molecular weight being about 500 000. According to *Yurchenko* and coworkers [13] the diameter of the fibre cross-section of myosin equals 2.2 nm. According to [14, 15] the myosin molecule consists of two heavy polypeptide chains of relative molecular weight of 200 000 and of four light chains, one having relative molecular weight 16 000, two chains 18 000, and one chain 20 000. The macromolecule is polar containing at one end two heads about 10 nm in diameter which interact with the actin [4].

Similarly F-actin belongs to proteins having a fibrillar structure. *Hanson* and *Lowy* [16] proposed, on the basis of electronmicroscopic picture, two models of F-actin fibres. The conclusions of their investigations are presented in [17]. The macromolecule of F-actin consists of tightly arranged identical spherical units about 5.5 nm in diameter. The F-actin fibre is formed by two together coiled macromolecules. Myosin polymerizes in salt solutions with F-actin giving rise to actomyosin. Actomyosin solutions show an intensive light scattering and a high viscosity.

According to our results the polymerization of myosin and F-actin results in a formation of two macromolecular aggregates. Yet it is not possible to draw the conclusions about their structure. It may, however, be supposed, as shown by *Huxley* [4] that the central actin fibril is "decorated" by myosin fibrils attached by means of heads and the rest of the fibril moves freely alongside the whole aggregate. The idea about two fractions present in the actomyosin solutions agrees with measurements of *Gergely* [18] and *von Hippel* [19] which similarly suggested a heterogeneous character of actomyosin solutions.

On the basis of the results obtained we may suppose that the method of small-angle X-ray scattering could be used also in the further investigations and possibly also for the investigation of pathological alterations of the quaternary structure of muscle proteins.

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