LETTERS TO THE EDITORS

Contraction and Relaxation of Actin Fibers

Since the discovery of actomyosin (AM) by Engelhardt, Szent-Györgyi, and others,[1] it has long been believed that an interaction between AM and ATP plays an essential role in muscle contraction. An AM fiber made by precipitation of a hypertonic AM solution into a physiological salt solution is contracted by ATP *in vitro*. Hayashi et al.[2] showed that a surface-spread AM fiber also is contracted by ATP, while a fiber of myosin made by the same method is not. Since the sliding or folding of thin filaments made of actin takes place at the contraction of the striated muscle and various possible mechanisms of such movement of actin have been proposed,[3-5] it is desirable to investigate the physical chemical properties of the actin fiber *in vitro*. However, to date, this kind of study has not been undertaken due to the difficulty of making the stable fiber.

The author succeeded in making an actin fiber by the technique developed by Hayashi and showed that the fiber is reversibly contracted by pH decrease or by adding neutral salts. Some denaturation seems to occur during the process of the fiber formation, because a similar fiber can also be formed from G-actin which was previously denatured by EDTA or anion exchange resin. Still it is interesting to note that the contractile fiber is made by actin alone.

MATERIAL AND METHODS

G-actin is prepared according to the method of Mommaerts.[6] A G-actin solution is spread on a surface of the salt-free water in the usual Langmuir trough, and after enough time for spreading, a surface monolayer of actin is compressed slowly by a movable strip. The fiber formed between strips is taken out and quickly fixed to a measuring apparatus and immersed in a medium (Tris buffer, 10 mM). Actin fibers can also be formed by spreading a G-actin solution or a F-actin solution on a surface of the salt solution buffered at neutral pH. Usually a fiber with diameter of about 100 microns and length of 20 mm. is used. The isotonic contraction of a fiber is carried out by hanging the fiber with small weight (1.3 mg.). Young's modulus of the fiber is measured by exerting a tension by sensitive torsion balance. In both cases, length changes of fibers are measured by a cathetometer.

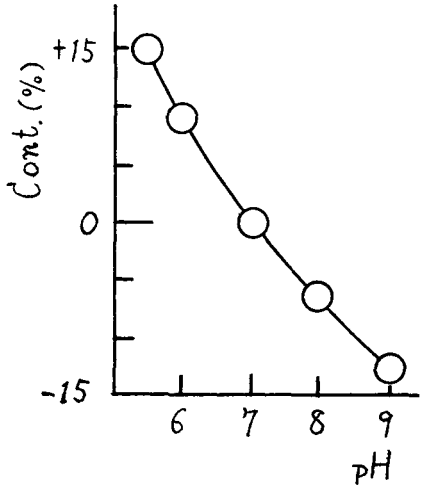

Fig. 1. Effect of pH changes on contraction and relaxation of actin fiber. Tris-maleate and Tris-HCl buffers (10 mM) were used.

RESULTS

The actin fiber contracts in low pH and reversibly relaxes in high pH as shown in Figure 1.

The fiber is also contracted by adding neutral salts (Fig. 2a) and relaxed by removing the salts. EDTA is a good relaxing reagent for the fiber contracted by divalent cations. The Young's modulus of the fiber increases in the contracted states (Fig. 2b), and its value (3×10^6–4×10^6 dyne/cm.^{2}) is the same as that of contracted native muscle.

At present, the contraction–relaxation of the actin fiber seems to be brought about by a polyelectrolytelike mecha-

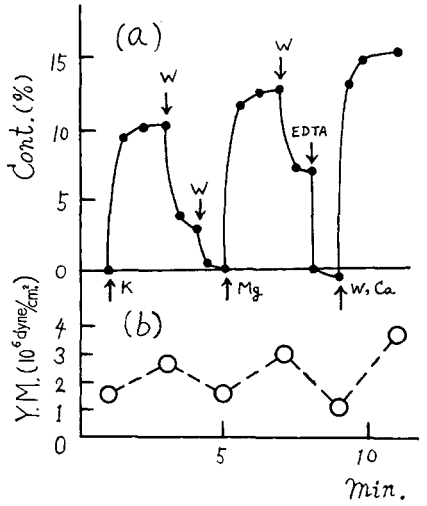

Fig. 2. Effect of repeated changes of salt concentrations at pH 7.0 on (a) contraction and relaxation of actin fiber, and (b) increase and decrease of Young's modulus of actin fiber (tris buffer 10 mM). Concentrations of the reagents: KCl 100 mM, $MgCl_2$ 5 mM, $CaCl_2$ 5 mM, and EDTA 1.5 mM. W indicates washing the fiber with the buffer.

nism. Fibers made by different procedures are now being compared.

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A Suggested Technique for Measuring Stress Relaxation Modulus and Creep Compliance and for Testing Linear Viscoelastic Theory

Stress relaxation is measured by subjecting a specimen at time zero to a suddenly applied strain, ϵ, which is thereafter held constant. The stress, σ, is measured as a function of time, and the quotient $\sigma(t)/\epsilon$ is the relaxation modulus, $G(t)$. Creep is measured by subjecting a specimen at time zero to a suddenly applied stress, σ, which is thereafter held constant. The strain, ϵ, is measured as a function of time, and the quotient $\epsilon(t)/\sigma$ is the creep compliance, $J(t)$.

In a stress relaxation experiment, it is difficult to measure the stress in the specimen without changing the strain, and most methods to date accept this disturbance but try to keep it negligibly small. In this letter, we shall show how some relations in the theory of linear viscoelasticity may be used to give the relaxation modulus and the creep compliance from data taken from a mixed system, by which we mean a system in which neither the stress nor the strain is constant.

Theory and Suggested Technique

In a linear viscoelastic material, the Laplace transforms of the stress and strain may be related through the transforms of either the relaxation modulus or the creep compliance, as follows:[1]

$$L\sigma(t) = sLG(t)L\epsilon(t) \tag{1}$$

$$L\epsilon(t) = sL\sigma(t)LJ(t) \tag{2}$$

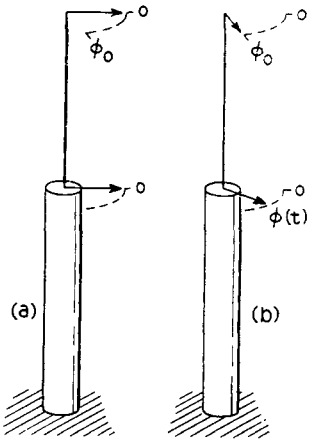

Fig. 1. (*a*) The unstressed system at $t < 0$. (*b*) The system at $t > 0$. The deformation φ_0 is held constant, and the other, $\varphi(t)$, is observed as a function of time.

where L indicates the transform. Also

$$LG(t)LJ(t) = 1/s^2 \tag{3}$$

Now let us consider the arrangement of Figure 1. A torsion wire of known relaxation modulus $G_1(t)$ is attached to the specimen whose unknown relaxation modulus $G_2(t)$ is desired. (It will be of advantage if the wire is as nearly perfectly elastic as possible, when $G_1(t)$ will be nearly constant.) The relaxation torsional stiffness, $M(t)$, of the wire of radius r is

$$M(t) = G_1(t)K_1/A_1$$

where

$$K = \pi r^4/2$$

and A denotes length. Similarly, that of the specimen is

$$N(t) = G_2(t)K_2/A_2$$

Then eq. (1) becomes

$$LT(t) = sLM(t)L\varphi(t)$$

where T is torque and φ is the twist in radians.

Before time zero, $\varphi_0 = \varphi = 0$, and the system is stress-free. At time $t = 0$, the upper end of the wire is suddenly twisted to φ_0, as a result of which the junction between the wire and the specimen starts a wandering angular history $\varphi(t)$. This is the quantity to be observed during the experiment. The twist in the wire is, then, $\varphi_0 - \varphi(t)$. The torque is the same in both wire and specimen, and its transform is, for the wire,

$$LT(t) = sLM(t)L[\varphi_0 - \varphi(t)] \tag{4a}$$

and for the specimen,

$$LT(t) = sLN(t)L\varphi(t) \tag{4b}$$

whence

$$LM(t)L[\varphi_0 - \varphi(t)] = LN(t)L\varphi(t) \tag{5}$$