Nano-Gel Machine Reconstructed from Muscle Proteins*

Kazuhiro Shikinaka, Akira Kakugo, Jian Ping Gong,† and Yoshihito Osada
Graduate School of Science, Hokkaido University, North 10, West 8, Sapporo 060-0810, Japan
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There are two basic differences in the motion between a human-made machine and a biological motor. One is in their principles. The motion of a human-made machine, which is constructed from hard and dry materials such as metals, ceramics or plastics, is realized by the relative displacement of the macroscopic constituent parts of the machine. In contrast to this, the motion of a living organism, which consists of soft and wet protein and tissues, is caused by the molecular deformation that is integrated to a macroscopic level through its hierarchical structure [1–3]. The other is in their energy sources. The human-made machine is fueled by electrical or thermal energy with efficiency around 30%, but a biological motor is driven by direct conversion from the chemical energy with efficiency as high as 80-90% [4]. In order to create biomimetic motility systems, polymer gels have been employed using their reversible size and shape change, thereby realizing the motion by integrating the deformation on a molecular level. Along this line, several kinds of artificial soft machines have been constructed using synthetic polymer gels in the past years. Gelooper (gel-looper), gelgolf (gel golf), gel valves, chemical motor, etc., are examples [5–11]. However, the lack of hierarchical structures and energy sources inside the gel lead to a decreased response and restricts the further application of such actuators for practical use in human bodies. Here we report an ATP fueled soft gel machine reconstructed from muscle proteins of actin and myosin. Chemically cross-linked actin gel filaments, several decade times the length of native actin filaments (F-actin) move along a chemically cross-linked myosin fibrous gel (1 cm long and 50 \( \mu \)m in diameter) with a velocity as high as that of native F-actin, by coupling to ATP hydrolysis. The motility observed in muscle protein-gels suggests that one might reconstruct a soft machine fueled by chemical energy by using actin and myosin molecules as elementary elements. [DOI: 10.1380/ejssnt.2005.51]

Keywords: Actin; Myosin; Gel; Soft machine

Actin gel formed from polymer-actin complexes

The actin molecule has an isoelectric point at pH 4.7, and is negatively charged at pH 6.8. We have found that the actin molecules formed complexes with cationic polymers, such as poly-(L-lysine) and x,y-ionene bromide polymer at pH 6.8, through electrostatic interaction to give filament-like aggregates (hereafter called, polymer-actin complexes). The polymer-actin complexes grew with time, and after several hours fibrous complexes of 10-50 \( \mu \)m in length (Fig. 1(b)), 5-10 times longer than the native F-actin (Fig. 1(a)), were observed under the fluorescence microscopy. As the lateral structures of the polymer-actin complexes are too small to be clearly observed by fluorescent optical microscope, we further studied polymer-actin complexes by transmission electron microscopy (TEM), using the negative staining technique. Figures 1(c) and 1(d) show the TEM images of native F-actin and the polymer-actin complex, respectively. As shown in Fig. 1(d), polymer-actin complexes form thin bundle. The large actin fibrous complexes were chemically cross-linked with transglutaminase (TG) to form stable gels (hereafter called, actin gel).

The motility assay of actin gel on the glass surface coated with myosin was performed with a geometry shown in Fig. 2. The chemically cross-linked actin gel showed sliding motion on the glass surfaces coated with myosin in spite of its large dimension (Fig. 4(a)).

Oriented myosin gel formed by shear stress

The chemically cross-linked myosin gel with its oriented filament array 1 cm long and 50 \( \mu \)m in diameter was obtained by reacting the scallop myosin at pH 7.0 using TG under stretching (refer to the methods section). The oriented myosin gel is semi-transparent, showing a swelling degree of c.a. 100, and a Young modulus of 190 Pa in the oriented direction, which is more than two times larger than that of the myosin gel prepared without stretching. Distinct bundles of regularly oriented filaments c.a. 1.5 \( \mu \)m in diameter are observed by using scanning electron microscopy (SEM: Fig. 3) and atomic force microscopy (AFM), indicating that the rod-like myosin molecules are self-organized with orientation to form a hierarchical structure. The molecular orientation of filaments was confirmed by the strong infrared (IR) dichroism of the carbonyl absorption at 1600 cm\(^{-1}\), which was not observed in the absence of stretching. Myosin molecules form bipolar filaments by self-assembly. So, it is considered that the direction of myosin molecules is not completely the same along the axis of the oriented myosin gel.

The chemically cross-linked myosin gel showed an ATPase activity as high as that of native myosin in the presence of 0.5 wt.-% native actin, which indicates that the active sides responsible for the interaction between actin and myosin were not destroyed by the cross-linking reaction.

F-actins showed a preferential motion along the axis of oriented myosin gel as elucidated by the degree of anisotropy (D.A.), which is defined as the ratio of the square-root average velocity in the fiber direction to that perpendicular to the fiber direction. The D.A. measured on the non-oriented myosin gel was 1.1(average over 66 samples), and that on the oriented myosin gel was 1.7(average over 91 samples). The mean velocity on the non-oriented myosin gel was 0.69 \( \mu \)m/s with a standard deviation of 0.24 \( \mu \)m/s, while that on the oriented myosin gel was 0.83 \( \mu \)m/s with a standard deviation of 0.30 \( \mu \)m/s.

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†also SORST, JST; Corresponding author: gong@sci.hokudai.ac.jp
Thus, F-actins prefer to move along the axis of the oriented myosin gel with an enhanced velocity.

Actin gel also showed a high motility on the oriented myosin gel. As shown in Fig. 4(b), the actin gel moved preferentially along the axis of oriented myosin gel almost without path deviations. The actin gels, about four times larger than native F-actin, moved with an average velocity of 1 $\mu$m/s, higher than that of native F-actins on the oriented myosin gel. Some of the actin gels moved as fast as 2.0 $\mu$m/s. In addition, D.A. of the actin gel was 2.2 (average over 38 samples) on the oriented myosin gel, which was higher than that of the native F-actin (D.A.=1.7), indicating an enhanced directional preference along the axis of the oriented myosin gel.

Thus, despite its increased mass, actin gel moves on the covalently cross-linked myosin gel, with an increased velocity. This means that the self-assembled and covalently bound actins and myosins can behave cooperatively, and exert a high motility coupling to ATP hydrolysis. This is rather surprising since the interaction between the myosin gel and the actin gel can only occur at the two-dimensional interface and due to cross-linking a considerable number of actin and myosin molecules are not involved in the sliding motion. Although the efficiency of the movement and the force generated during the sliding motion are not measured here, the described muscle protein-gels suggest that one might build up human-made machines fueled by chemical energy by using actin and myosin molecules as elementary elements. This kind of protein gel machine with desired shape, size, and function might run in human body without causing any immunoreactions if it is reconstructed from the protein molecules of the same body.

METHODS

Materials

Myosin was obtained from scallops by the BARANY method [12]. Actin was obtained from scallops by the method of Spudich et al. [13].

Myosin gel

Myosin (10 mg/ml) dissolved in 50 mM MOPS (pH 7.0) containing 600 mM KCl was spread on the slide glass plate and then dipped into 50 mM MOPS (pH 7.0) containing 10 mM KCl for 10 min. at 4°C keeping the angle of slide glass to water surface as 60° to the water surface. Using this method, globular myosin transfers to myosin filament to form a cloudy film on the buffer surface. Part of the film edge was slowly picked up.
using tweezers, stretched with a velocity of 3-4 mm/s, and dipped in TG solution (1 unit against 1 mg myosin) for 30 min at 13°C to give chemical cross-links. The cross-linked myosin filament was rinsed in 50 mM MOPS (pH 7.0) containing 600 mM KCl for several hours to remove soluble myosin.

**Actin gel**

The chemically cross-linked actin gel was obtained from actin solution (0.07 mg/ml) containing stoichiometric concentration of rhodamine-phalloidin (Molecular Probes no. 4171), 80 mM KCl, 3 mM MgCl₂, 40 mM KH₂PO₄ (pH 6.8), and 1.5 unit/mg TG as the cross-linker in the presence of poly(L-lysine) (100 unit/mol). After cross-linking at 20°C for 1 h, the actin gels were sonicated and dialyzed against 2 mM Tris (pH 8.0), 0.2 mM CaCl₂, 0.2 mM ATP, 0.5 mM 2-mercaptethanol to dissociate the physically polymerized actin.

**Mg-ATPase activity**

The actin activated Mg-ATPase activity was measured by determining the amount of inorganic phosphate liberated from ATP for 8 min at 15°C (50 mM KCl, 20 mM Tris (pH 7.4) containing 2 mM MgCl₂, 0.3 mM CaCl₂ and 1 mM ATP) in the presence of myosin gels (0.66 mg/ml) and 0.5 wt-% native actin, or actin gels in the absence of monomeric actins and myosins. The reaction was terminated by adding 10% of trichloroacetic acid (TCA). The liberated inorganic phosphate was determined by the method of Youngburg and Youngburg [14].

**Electron microscopy**

TEM was performed by using a JEOL (JEM-1200EX) at 120 kV acceleration voltage. A drop of F-actin-polymer mixture of about 10 µl was put on carbon-coated 200 mesh grids that were rendered hydrophilically by glow discharge in a reduced pressure. After waiting for 180 s for adsorption, the grids were stained by one drop of phosphotungstic acid (pH 7.2).

**Motility assay** [15–17]

Myosin was coated on a cover glass surface, which was pre-coated with 0.03% nitrocellulose. The cover glass was then placed on a slide glass equipped with spacers 1.1-1.4 mm high placed at both sides (Fig. 2). The flow-cell made in this way was filled with 50 mM MOPS (pH 7.0) buffer and placed on ice. The flow-cell was washed with 600 mM KCl and 50 mM MOPS (pH 7.0) and then was filled with buffer (50 mM KCl, 25 mM DTT, 2 mM MgCl₂, 0.3 mM CaCl₂ and 30 mM imidazole (pH 7.6) containing 0.3 wt-% methylcellulose. 2 µl of actin (10 µM) labeled with rhodamine-phalloidin in assay buffer was introduced into the flow-cell. The cell was placed on the stage of a fluorescence microscope (Olympus BX 50) and the fluorescent-labeled actins were observed under a ×60 objective. The movement of F-actin or actin gel was initiated by filling the cell with assay buffer containing prescribed amount of ATP concentration at 20°C. The fluorescent image was obtained with an inverted CCD-camera (Olympus Color Camera HCC-3900) and recorded on a videotape recorder (Digital Videocassette Recorder DSR-30). The velocity of native F-actins or actin gels on myosin gel was evaluated by the displacement of their center position in 3 s. The motility assay on oriented myosin gel was carried out in the same method. The effect of the fiber orientation of the myosin gel on the direction of actin movement was evaluated by the degree of anisotropy (D.A.), which is defined...
FIG. 4: Sequential fluorescence microscope images of the motion of actin gel consisted of poly-lysine-actin complex on the glass surface coated with myosin (a) and on the oriented myosin gel (b). Scale bar is 10 µm.

as follows:

\[ D.A. = \frac{\bar{x}}{\bar{y}} \]

where,

\[ \bar{x} = \frac{\sum_{i=1}^{N} x^2_i}{N}, \quad \bar{y} = \frac{\sum_{i=1}^{N} y^2_i}{N}. \]

Here \( N \) is the number of samples, and \( x_i \) and \( y_i \) are the respective components of displacement parallel and vertical to the myosin fiber axis in 3 s of the \( i \)-th sample.

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