The "Le Chatelier's Principle"-Governed Response of Actin Filaments to Osmotic Stress

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Actin filaments inhibit osmotic stress-driven water flow across a semipermeable membrane in proportion to the filament concentration (Ito, T.; Zaner, K. S.; Stossel, T. P. *Biophys. J.* **1987**, *51*, 745). When the filaments are cross-linked by F-actin binding protein, filamin A, this flow is stopped completely (Ito, T.; Suzuki, A.; Stossel, T. P. *Biophys. J.* **1992**, *61*, 1301). No conventional theory accurately accounts for these results. Here, this response is analyzed by formulating the entropy of the system under osmotic stress. Results demonstrate that the response of the actin filaments to osmotic stress is governed by the Le Chatelier's principle, which states that an external interaction that disturbs the equilibrium brings about processes in the body that tend to reduce the effects of this interaction. In the present case, disrupting equilibrium by osmotic stress brings about a reaction that decreases the chemical potential of water in the F-actin solution, reducing the effect of the applied osmotic disturbance. This decrease in the chemical potential of the water in the F-actin solution is caused by an increase in the chemical potential of F-actin, which is induced by isothermal absorption of heat by F-actin aided by work done by osmotic stress. As a result, F-actin has an inhibitory effect on the osmotic stress-driven water flow, and can even completely stop the flow when it is cross-linked. This is the first report demonstrating that the Le Chatelier's principle applies to the reaction of biopolymers against equilibrium disturbances such as osmotic stress.

1. Introduction

The periphery of many eukaryotic cells contains a network composed of actin filaments (F-actin). Actin networks have certain mechanical properties in response to imposed forces. Many studies have investigated the mechanical properties of F-actin networks both in vivo and in vitro. Measurements of cell deformation in response to an imposed force reveal that actin networks in the cell cortex possess viscoelastic properties.[1,2] The in vitro approaches using F-actin solutions and reconstituted actin networks show that such viscoelastic properties strongly depend on length, concentration, and degree of F-actin cosslinking with higher concentration and longer length bestowing more elasticity.[3,4]

Cells regulate volume under osmotic stress across the cell membrane primarily by adjusting membrane permeability to water and ions. Another dimension worth considering is that actin filament networks in cell periphery may play a role in the reaction of cells to osmotic stress, since networks of synthetic polymers react to osmotic stress to equilibrate it by changing their configurations.[5−7] Unlike a response to mechanical forces, however, the reaction of biopolymer networks to osmotic stress has not been widely studied despite its importance. No quantitative studies on water flow through biopolymer networks induced by osmotic stress have been released except from our laboratory.

Previously, we demonstrated that F-actin retarded osmotically driven water flow across a semipermeable membrane, using an osmometer containing F-actin or actin networks separated from the outside-buffered bath by a semipermeable membrane.[8,9] Two types of inhibitory effects were observed. One, evident in un-cross-linked F-actin solutions, was proportional to the F-actin concentration but was independent of the polymer length. The second type was operative in networks of actin filaments cross-linked by filamin A, which is a high molecular weight homodimer that promotes perpendicular branching of F-actin. In these networks, water flow stopped completely under the same osmotic stress that only slowed the flow in an un-cross-linked F-actin solution of the same actin concentration (>0.5 mg/mL). Although experimental results were unmistakable, no conventional theory could explain why and how actin filaments react to osmotic stress in this manner.

In this report, we elucidate the mechanism of the inhibitory effect of F-actin on osmotic stress-induced water flow on the basis of nonequilibrium thermodynamics, addressing a novel problem of how osmotic stress changes the chemical potential of the F-actin. The osmotic stress imposed across a semipermeable membrane helps F-actin to absorb heat from external medium isothermally. As the result, F-actin may increase its chemical potential to reduce the effect of the applied osmotic disturbance as predicted by the Le Chatelier's principle, which states that an external interaction that disturbs the equilibrium brings about processes in the body which tend to reduce the effects of this interaction. This kind of effect of osmotic stress has not been reported up to the present, even though the phenomenon of osmosis has been studied widely since the age of van't Hoff more than one hundred years ago.

2. Results

2.1. Formulating Entropy of Osmometer System. *Experimental System.* Figure 1a is a schematic representation of the

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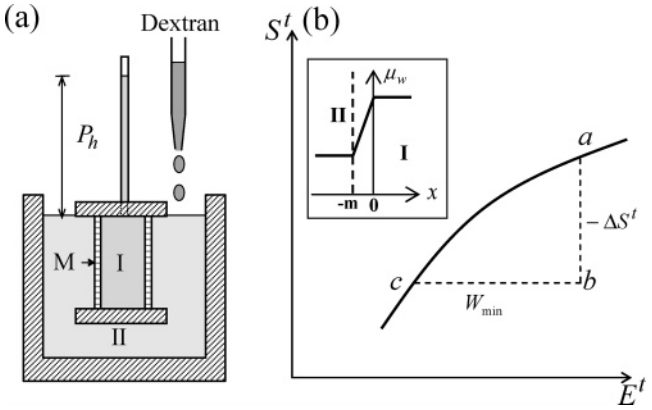

Figure 1. (a) Schematic representation of the experimental system of Ito et al:[8,9] (I) sample solution ("body", ~1.5 mL) in an osmometer vessel with a 0.9 mm-diameter capillary; (II) outside buffer bath ("medium", ~500 mL); (M) molecular weight cutoff semipermeable membrane; (P_h) equilibrium hydrostatic pressure; (Dextran) membrane-impermeable dextran added to II to create osmotic stress. Panel b shows entropy, S^t, of the entire system illustrated in panel a as a function of energy, E^t. The continuous line represents the equilibrium entropy. The ba segment of the vertical hatched line corresponds to the difference in entropy ΔS^t between the nonequilibrium state under consideration, denoted by point b, and the final equilibrium state, denoted by point a. The bc segment of the horizontal hatched line corresponds to the minimum work, W_{min}, done on the body by a thermally isolated source of work ("external source") to bring the system to a nonequilibrium state at point b. See the text for details. The inset is a profile of the chemical potential of water in the stationary state. The chemical potential is plotted as a function of position. Compartment I, which is inside of the osmmeter, is $x > 0$. The membrane itself is $-m \le x \le 0$. Compartment II, which is the outside buffer bath, is $x < -m$.

experimental system by Ito et al.[8,9] The system is composed of two subsystems, that is, the F-actin solution, or "body", in the osmometer vessel (compartment I in Figure 1a), and the outside buffer bath, or "medium" (compartment II). The F-actin solution is separated from the outside by a high molecular weight cutoff semipermeable membrane (M). In the experiments, the entire system (body and medium) is initially in equilibrium under ~20 cm·H_2O of hydrostatic pressure P_h provided by 20 mg/mL of membrane-impermeable dextran (~40 kD) included in the F-actin solution in compartment I. The equilibrium is then destroyed by the addition of dextran to the outside buffer bath in compartment II to induce osmotic stress P_f on the F-actin solution, represented by

$$P_f = P_h - RT\Delta C \tag{1}$$

where ΔC is the dextran concentration difference between compartment I and II. The P_f value is adjusted by varying the concentration of dextran in compartment II. This equilibrium disruption induces water flow from compartment I to II, which is measurable quantitatively from the meniscus change in the capillary. The system reaches a stationary state within 10 min, accompanied by stationary water flow, J_w. Finally, the entire system attains a new equilibrium state when water flow ceases at $P_f = 0$.

Profile of the Chemical Potential of Water. The inset in Figure 1b diagrams the chemical potential profile of water in the stationary state. The gradient of the chemical potential localizes only in the membrane, indicating that the F-actin solution is thermodynamically homogeneous on the basis of the following analysis. Stirring of the solution in compartment II rapidly dissipates the dextran concentration gradient to eliminate the chemical potential gradient in the water in compartment II. The membrane used is assumed to be homogeneous so that the chemical potential gradient of the water is constant across the membrane. The observed velocity of the osmotic stress-induced water flow J_w was very slow, on the order of several tens of μl/hr, so that the pressure is equilibrated adequately and does not produce a velocity gradient in the F-actin solution in compartment I. Consequently, no viscous force is expected during water flow, because a viscous force stems from a velocity gradient. In this case, J_w is equal to $(\phi_w/f_w)(d\mu_w/dx)$,[10] where ϕ_w, f_w, and $d\mu_w/dx$ represent the volume fraction of water, the frictional coefficient of a water molecule, and the chemical potential gradient of water, respectively. Since the flow must be continuous at the boundary between the membrane and compartment I, it follows that

$$\left(\frac{d\mu_w(0)}{dx}\right)^I = \frac{\phi_w^m/f_w^m}{\phi_w^I/f_w^I}\left(\frac{d\mu_w(0)}{dx}\right)^m \tag{2}$$

where $(d\mu_w(0)/dx)$represents the gradient at the boundary, and the superscripts m and I denote the values in the membrane and in compartment I, respectively. The gradient in compartment I should have a maximum value at the boundary because the gradient is made by changing the osmolality in compartment II. Therefore

$$\left(\frac{d\mu_w(x)}{dx}\right)^I \le \frac{\phi_w^m/f_w^m}{\phi_w^I/f_w^I}\left(\frac{d\mu_w(0)}{dx}\right)^m \tag{3}$$

where $(d\mu_w(x)/dx)^I$ is the gradient at an arbitrary point in compartment I. To evaluate the value of $(d\mu_w(x)/dx)^I$ from eq 3, the values of ϕ_w^m/f_w^m and ϕ_w^I/f_w^I are needed. The inverse of the value of ϕ_w^m/f_w^m for the membranes used is 3.3×10^{13} dyn·sec/cm·mol obtained from the experimental values of the membrane filtration coefficient, $L_P = J_w/P_f = \phi_w^m\bar{V}_w/f_w^m\Delta x$, using 90 μm as membrane thickness Δx as reported by the manufacturer. The value of f_w^I is calculated as follows. Onsager's law states that the ratio of the frictional coefficient on a solute molecule f_s to that on a solvent molecule f_w is equal to the inverse of the molar ratio of the solute molecules to the solvent molecules, m, that is, $f_w = mf_s$. Stoke's law states that $f_s = 6.02 \times 10^{23} \times 6\pi r\eta$, where r and η represent the radius of the solute molecule and viscosity of the solvent, respectively. In a solution of 1 mg/mL G-actin, taking 2 nm as the value of r, f_w is 1×10^{10} dyn·sec/cm·mol. The value of f_w for an F-actin solution must be smaller since the contact area of a G-actin molecule with water is larger than that of a subunit molecule in an F-actin filament. The value of ϕ_w^I is assumed to be unity in solution. Therefore, the following relation can be assumed at 1 mg/mL F-actin:

$$\left(\frac{d\mu_w(x)}{dx}\right)^I \le 3.0 \times 10^{-4}\left(\frac{d\mu_w(0)}{dx}\right)^m \tag{4}$$

Equation 4 indicates that the gradient at 1 mg/mL F-actin is <0.03%, and at 3.5 mg/mL is <0.1% of the gradient in the membrane, a negligible amount considering the concentration range used in these experiments. Therefore, the chemical potential of water in the stationary state is like the one shown in the inset of Figure 1b, in which the gradient produced by the osmolality change exists only in the membrane and not in

compartment I or II. These results indicate that the solution in compartment I is in a thermodynamically homogeneous state, where neither viscous nor frictional forces are at work.

Description of the Thermodynamic States. The results described above allow a thermodynamic analysis of the experimental data on J_w. Figure 1b is a diagram of the thermodynamic states of the entire system (I and II in Figure 1a), which is considered a thermally isolated, closed system with a constant volume. The vertical and horizontal axes correspond to the total entropy S^t and internal energy E^t of the entire system, respectively. The continuous line represents S^t at equilibrium, which is a function of E^t. Point b corresponds to the nonequilibrium state, which the entire system reaches just after disruption of osmotic balance by addition of dextran. At point b, the body (i.e., the F-actin solution in compartment I) is not in equilibrium with the medium (i.e., the outside buffer bath in II) or with itself, and the total entropy differs from its equilibrium value $S^t(E^t)$ for the same value of the total energy E^t by some amount $\Delta S^t < 0$. Point a represents the final equilibrium state, which the system reaches spontaneously along vertical line ba, accompanied by flow of water from the inside to the outside of the osmometer. In this case, segment ba corresponds to $-\Delta S^t$. Point c on the continuous line of equilibrium is a theoretically defined state, which is introduced as a crossing point between the equilibrium line and the horizontal line through point b. It should be noted that the initial state of the experiments, which is in equilibrium prior to the dextran addition, does not exist on this equilibrium line.

Evaluation of the Entropy. The ΔS^t was formulated according to the method of Landau and Lifshitz.[11] The first assumption is that the entire system is in equilibrium initially at some point on the continuous equilibrium line shown in Figure 1b (note that this state does not represent the "actual" equilibrium state before the addition of dextran), and then proceeds to a nonequilibrium state at point b in Figure 1b through work W on the body done by an "external source" which is thermally isolated from both the medium and body. During the process, the body receives an amount of energy ΔQ from the medium, and increases its internal energy by ΔE^i. From the first law of thermodynamics

$$W = \Delta E^i - \Delta Q \tag{5}$$

In the present case, the body is the F-actin solution in the osmometer vessel and the medium is the outside buffer bath. Under the condition that temperature, T is a constant, ΔQ and W are represented by

$$\Delta Q = -T\Delta S^o + P^o\Delta V^o - \mu_w^o\Delta n_w^o = -T\Delta S^o - P^o\Delta V^i + \mu_w^o\Delta n_w^i \tag{6}$$

$$W = \Delta E^i - \Delta Q = \Delta E^i + T\Delta S^o + P^o\Delta V^i - \mu_w^o\Delta n_w^i \tag{7}$$

where μ_w and n_w are chemical potential and number of molecules of water, respectively, S and V are entropy and volume, respectively, and the suffixes i and o represent the inside and outside of the osmometer, respectively. The entire system with the F-actin solution as the body plus the outside buffer bath as the medium can be regarded as a thermally isolated closed system. The law of increase of entropy gives $\Delta S^i + \Delta S^o \geq 0$, which enables derivation of the minimum work, W_{min} by replacing ΔS^o with $-\Delta S^i$ in Equation 7:

$$\begin{aligned} W \geq W_{min} &= \Delta E^i + P^o\Delta V^i - T\Delta S^i - \mu_w^o\Delta n_w^i \\ &\approx \Delta E^i + P^i\Delta V^i - T\Delta S^i - \mu_w^o\Delta n_w^i \\ &= \Delta(E^i + P^iV^i - TS^i) - V^i\Delta P^i - \mu_w^o\Delta n_w^i \\ &= \Delta G^i - V^i\Delta P^i - \mu_w^o\Delta n_w^i \\ dW_{min} &= dG^i - V^i\,dP^i - \mu_w^o\,dn_w^i \end{aligned} \tag{8}$$

where an approximation of $P^o\Delta V^i = P^i(1 - P_h/P^i)\Delta V^i \approx P^i\Delta V^i$ is involved, because $P_h/P^i \approx 0.01 \ll 1$ in the present case.

W_{min} in the above equation corresponds to segment cb in Figure 1b, and ΔG^i is the change in Gibbs free energy G^i of the F-actin solution along $c \rightarrow b$. As G^i is a homogeneous function of the first-order in the moles of water and F-actin in the body, n_w^i and n_f, respectively, the equation of $G^i = n_w^i\mu_w^{i\prime} + n_f\mu_f$ holds, where $\mu_w^{i\prime}$ and μ_f are the chemical potentials of water and F-actin in the body, respectively. Thus,

$$\begin{aligned} dW_{min} &= d(n_w^i\mu_w^{i\prime} + n_f\mu_f) - V^i\,dP^i - \mu_w^o\,dn_w^i \\ &= (\mu_w^{i\prime} - \mu_w{}^o)\,dn_w^i + n_w^i\,d(\mu_w^{i\prime} - \bar{V}_wP^i) + n_f\,d\mu_f \\ &= \Delta\mu_w\,dn_w^i + n_w^i\,d\mu_w^i + n_f\,d\mu_f \end{aligned} \tag{9}$$

where $\bar{V}_w$ is the molar volume of water, and ΔP^i is the pressure change. In eq 9, $\Delta\mu_w$ ($\Delta\mu_w = \mu_w^{i\prime} - \mu_w^o$) represents the chemical potential difference of water between the body and medium, and $d\mu_w^i$ [$d\mu_w^i = d(\mu_w^{i\prime} - \bar{V}_wP^i)$] corresponds to the pressure-independent term of total differential of the chemical potential of water in the body,[12] and $V^i \cong n_w^i\bar{V}_w$.

Since the body (~1.5 mL) is a very small part of the entire system (~500 mL), the shift of the entire system along $c \rightarrow b \rightarrow a$ causes only a negligible change in total energy and entropy. Therefore, Figure 1b shows that

$$\Delta S^t = -(dS^t/dE^t)W_{min} = -(1/T)W_{min} \tag{10}$$

since the slope of the continuous line represents the inverse of the temperature. For an infinitesimal change in the state of the entire system

$$dS^t = -(1/T)\{\Delta\mu_w\,dn_w^i + n_f[1 + (n_w^i/n_f)(d\mu_w^i/d\mu_f)]\,d\mu_f\} \tag{11}$$

The condition $\Delta S^i + \Delta S^o = 0$ does not necessarily mean that the body is always in equilibrium at every point along line cb in Figure 1b. The necessary condition of such an equilibrium process is that the relation representing the reversible change of the body, that is, the Gibss−Duhem relation of $n_w^i\,d\mu_w^{i\prime} + n_f\,d\mu_f - V^i\,dP^i = 0$ holds at every point on which the chemical potentials of water and F-actin are $\mu_w^{i\prime}$ and μ_f, respectively, bringing the second term of the right side in eq 11 to zero, because $n_w^i\,d\mu_w^{i\prime} + n_f\,d\mu_f - V\,dP^i = n_w^i\,d(\mu_w^{i\prime} - \bar{V}_wP^i) + n_f\,d\mu_f = n_w^i\,d\mu_w^i + n_f\,d\mu_f = 0$. Hereafter, we shall represent the chemical potential of water in the body as μ_w^i by dispensing with the dash for simplicity, and apply the relation of $n_w^i\,d\mu_w^i + n_f\,d\mu_f = 0$ to the equilibrium condition in the body without expressing the pressure term explicitly unless necessary.

2.2. Effects of F-Actin on Stationary Water Flow. Figure 2 summarizes the experimental results of the stationary water flow J_w from the F-actin solutions at various intensities of

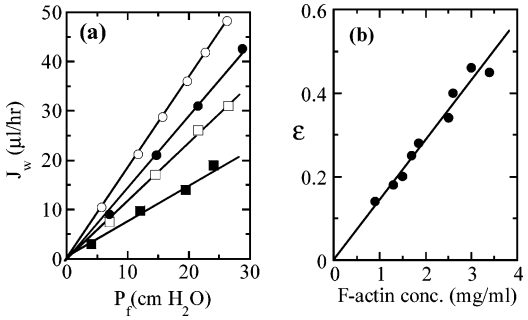

Figure 2. (a) Dependence of osmotic stress-induced water flow on F-actin concentration. The water flow J_w at various F-actin concentrations [0 mg/mL (○), 1.0 mg/mL (●), 2.0 mg/mL (□), and 3.3 mg/mL (■)] is plotted against osmotic stress P_f. (b) Dependence of ϵ on F-actin concentrations is shown. The individual values of ϵ are derived from the slope of P_f versus J_w according to eq 12. From the data of Ito et al.[8,9]

osmotic stress P_f obtained by Ito et al.[8,9] As evident in the figure, the following relation holds between J_w and P_f:

$$J_w \propto (1 - \epsilon)P_f \tag{12}$$

$$\epsilon \propto C_f \tag{13}$$

where C_f is the mass concentration of F-actin. Nonfilamentous actin (G-actin) or dextran used in the experiments had no effect on the water flow at any concentration tested ($\leq$10 mg/mL for G-actin, $\leq$20 mg/mL for dextran). These results were highly reproducible even for membranes that were used repeatedly (more than five times).

As discussed in section 2.1, no viscous force is expected to work during the osmotically driven water flow, and therefore the inhibitory effects of F-actin on J_w cannot be attributed to the viscous properties of the solution. In fact, the inhibitory effects did not depend on the diameter of the osmometer capillary and its surface properties. Changing the diameter of the glass capillary from 0.9 to 1.3 mm had no effect on J_w, nor did treating the capillary with siliconizing reagent, which may make the capillary surface hydrophobic instead of hydrophilic. In addition, the inhibitory effect was independent of filament length from $\sim$10 μm to $\sim$0.2 μm, while the falling-ball viscosity of the solution decreased several 100-fold. These results indicate that the viscous properties of the F-actin solution cannot account for the inhibitory effect on J_w.

Given the above argument, the effect of the actin filaments must be attributed to thermodynamics. In the present experiments, the chemical potential gradient of water is localized through the semipermeable membrane, with little existing in the F-actin solutions, as shown in the inset of Figure 1b. In this case, the stationary water flow J_w is driven by the chemical potential gradient of water through the semipermeable membrane, which is proportional to the difference in chemical potential of water between the inside (μ_w^i) and outside (μ_w^o) of the osmometer.[10] Hence, the following relation is derived from the empirical formula in Equation 12:

$$\Delta\mu_w = \mu_w^i - \mu_w^o = \bar{V}_w(1 - \epsilon)P_f \tag{14}$$

where $\bar{V}_w$ is the partial molar volume of water.

2.3. Thermodynamic Interpretation of F-Actin Effects. Equation 14 shows that the inhibitory effect of F-actin on osmotic stress-induced water flow is due to a decrease in the chemical potential of water in the F-actin solution,[8] indicating that the chemical potential of water in the F-actin solution may be affected by the addition of membrane-impermeable dextran to the outside buffer bath separated by the membrane. How is the chemical potential of water affected without any direct interaction between the dextran molecules added and the F-actin solution? No conventional theory is available to explain this. We shall interpret it by applying the entropy formula of eq 11, using the notation $x \equiv n_w^i$, $y \equiv \mu_f$, and

$$X \equiv -\partial S^t/\partial x = (1/T)\Delta\mu_w \tag{15}$$

$$Y \equiv -\partial S^t/\partial y = (1/T)n_f[1 + n_w^i/n_f(\mathrm{d}\mu_w^i/\mathrm{d}\mu_f)] \tag{16}$$

As described under eq 11, $Y = 0$ is the condition of internal equilibrium of the F-actin solution itself, and $X = 0$ and $Y = 0$ are necessary conditions for equilibrium of the entire system. As S^t has its *maximum value* in equilibrium of the entire system, the following conditions apply:[11]

$$(\partial X/\partial x)_y > 0, \quad (\partial Y/\partial y)_x > 0,$$
$$(\partial X/\partial x)_y(\partial Y/\partial y)_x - (\partial X/\partial y)_x^2 > 0 \tag{17}$$

Using the properties of Jacobians and the inequalities in eq 17, we have

$$(\partial X/\partial x)_{Y=0} \equiv \frac{\partial(X,Y)}{\partial(x,Y)} = \frac{\partial(X,Y)/\partial(x,y)}{\partial(x,Y)/\partial(x,y)} =$$
$$(\partial X/\partial x)_y - (\partial X/\partial y)_x^2/(\partial Y/\partial y)_x > 0,$$
$$(\partial X/\partial x)_y > (\partial X/\partial x)_{Y=0} > 0 \tag{18}$$

Here, let us disrupt the equilibrium in the entire system by adding membrane-impermeable dextran to the outside buffer bath. The entire system enters a nonequilibrium state at point b in Figure 1b. The quantity $x \equiv n_w^i$ deviates from the equilibrium value by $\Delta x = \Delta n_w^i > 0$, and the condition $X = 0$ is no longer satisfied, while $y \equiv \mu_f$ is not directly affected by upsetting the osmotic balance. The change in X at the instant of disruption of the equilibrium is then

$$(\Delta X)_y = (\partial X/\partial x)_y\Delta x = (1/T)(\partial\Delta\mu_w/\partial n_w^i)_y\Delta n_w^i \tag{19}$$

The change in x at constant y leads to a violation of the condition $Y = 0$, that is, of the internal equilibrium of the F-actin solution. When the internal equilibrium is restored again, $X \equiv \Delta X$ will be

$$(\Delta X)_{Y=0} = (\partial X/\partial x)_{Y=0}\Delta x = (1/T)(\partial\Delta\mu_w/\partial n_w^i)_{Y=0}\Delta n_w^i \tag{20}$$

where $\Delta\mu_w$ is the deviation from the equilibrium value at point c in Figure 1b. From eqs 18−20,

$$(\Delta X)_y > (\Delta X)_{Y=0}$$
$$(\Delta\mu_w)_y = (\partial\Delta\mu_w/\partial n_w^i)_y\Delta n_w^i > (\partial\Delta\mu_w/\partial n_w^i)_{Y=0}\Delta n_w^i =$$
$$(\Delta\mu_w)_{Y=0} \tag{21}$$

The equilibrium state at point c is a theoretically introduced one, and so the value of the chemical potential at this point needs to be evaluated by experimentally attainable values. Since the temperature at point c is nearly equal to that at point a under constant volume, the equilibrium pressure should little change along c $\rightarrow$ a, so that $\mathrm{d}\mu = -\bar{S}\,\mathrm{d}T + \bar{V}\,\mathrm{d}P^o \sim 0$. Therefore, the individual value of the chemical potential at point c is similar to that at point a. Thus, from eq 21

$$(\Delta\mu_w)_y = \bar{V}_w P_f > (\Delta\mu_w)_{Y=0} \quad (22)$$

where P_f is the applied osmotic stress needed to disrupt the osmotic balance, which is accomplished by the addition of membrane-impermeable dextran to the outside buffer bath, as expressed by eq 1. The value $(\Delta\mu_w)_y$ in eq 22 is the difference in water chemical potential between the F-actin solution and that of the outside buffer bath just after disruption of the osmotic balance, and $(\Delta\mu_w)_{Y=0}$ is the difference after restoring the internal equilibrium of the F-actin solution. Equation 22 forms the content of the Le Chatelier's principle, which states that an external interaction that disturbs the equilibrium brings about processes in the body, which tend to reduce the effects of this interaction. In the present case, the disturbance caused by osmotic stress triggers a process that decreases the chemical potential of water in the F-actin solution to reduce the effect of the disturbance (eq 14).

This decrease in the chemical potential of water in the F-actin solution relates to an increase in the chemical potential of F-actin as follows. The water flow in Figure 2 is stationary flow from the F-actin solution after the internal equilibrium is restored, but equilibrium with the external environment is not (i.e., $Y = 0$, $X \neq 0$). The condition $Y = 0$ leads to

$$n_w^i \, d\mu_w^i + n_f \, d\mu_f = 0 \quad (23)$$

so that

$$n_w^i \Delta\mu_w^i + n_f \Delta\mu_f = 0 \quad (24)$$

where $\Delta\mu_w^i$ and $\Delta\mu_f$ are deviations in the chemical potentials of water and the F-actin in the F-actin solution from those in the overall equilibrium state (i.e., $X = 0$, $Y = 0$), respectively.[13] Note that $\Delta\mu_w^i$ in eq 24 corresponds to the pressure-independent part in total change of the chemical potential. Thus, from eqs 22 and 24, the difference in the chemical potential of water between the inside and outside of the osmometer in the stationary state, $(\Delta\mu_w)_{Y=0}$ can be described as

$$(\Delta\mu_w)_{Y=0} = \bar{V}_w P_f + \Delta\mu_w^i = \bar{V}_w(1 - [n_f]\Delta\mu_f/P_f)P_f = \bar{V}_w(1 - \epsilon)P_f \quad (25)$$

where $0 < \epsilon = [n_f]\Delta\mu_f/P_f < 1$, and $[n_f]$ is the concentration of F-actin.

Equation 25 expresses that the decrease in the chemical potential of water in the stationary state relates directly to an increase in the chemical potential of F-actin. Hence, it is reasonable to consider that the increase in the chemical potential of F-actin is induced by the osmotic stress, and, as a consequence of this, the chemical potential of water decreases in the stationary state.

A linear relationship between $\Delta\mu_f$ and P_f may hold in the stationary state near equilibrium, so that ϵ in eq 25 is constant at a constant concentration of F-actin, and therefore a linear relation holds between J_w and P_f, as experimentally demonstrated in Figure 2a. In addition, ϵ is linearly proportional to the mass concentration of F-actin (Figure 2b), since the chemical potential of F-acin is proportional to the filament length that is inversely proportional to the filament number, resulting in that $\Delta\mu_f$ in eq 25 is independent of the mass concentration of F-actin.

2.4. Mechanism of Chemical Potential Change of F-Actin. The results in the analysis of section 2.3 indicate that the applied osmotic stress stimulates some process in the F-actin solution that increases the chemical potential of the F-actin by $\Delta\mu_f$ at constant pressure and temperature. In this case, the change in Gibbs free energy, ΔG^i, of the F-actin solution is equal to $n_f\Delta\mu_f$. Since

$$H^i = G^i + TS^i = -T^2\left(\frac{\partial}{\partial T}\frac{G^i}{T}\right)_{P,}$$

$$\Delta H^i = -T^2\left(\frac{\partial}{\partial T}\frac{\Delta G^i}{T}\right)_P = -T^2\left(\frac{\partial}{\partial T}\frac{n_f\Delta\mu_f}{T}\right)_P = -T^2\left(\frac{\partial}{\partial T}\frac{\epsilon P_f}{T}\right)_P = \epsilon P_f - T\frac{\partial\epsilon}{\partial T}P_f \quad (26)$$

where ΔH^i is the change in the enthalpy of the F-actin solution. The values of ϵ of 2.0 mg/mL F-actin solution measured at 25 and 37 °C are 0.25 and 0.21, respectively. Using 0.25 as the ϵ value with the approximation of $\partial\epsilon/\partial T \approx -0.0033$ in eq 26 allows the estimation of ΔH^i at 25 °C as follows:

$$\Delta H^i = 1.25P_f = 5n_f\Delta\mu_f \quad (27)$$

Osmotic stress is produced by the addition of membrane-impermeable dextran to the outside solution. In this case, no change in the solvent-filament interactions or in the interfilament interactions in the inside of the osmometer is expected, because no direct contact of the dextran with the actin filaments or solvent occurs. In fact, shortening the filament length from 10 to 0.2 μm does not change the effects of osmotic stress, although it must significantly decrease the interfilament interactions. In addition, when an F-actin solution is subjected to the osmotic stress of up to 50 cm·H_2O, no change occurs in the light scattering that is sensitive to changes in interfilament interactions. These results indicate that a change in interfilament interactions may not trigger the response of actin filaments to osmotic stress. Thus, the enthalpy change, ΔH^i in eq 27 must be attributed to an increase in the internal energy of the single filaments.

2.5. Osmotic Stress-Activated Process to Increase Chemical Potential of F-Actin. As described, thermodynamics favors an increase in the chemical potential of F-actin in response to osmotic stress. However, the process through which osmotic stress induces the increase in the chemical potential of F-actin has not been discussed. Briefly, osmotic stress does some work on the F-actin solution that helps F-actin to absorb heat isothermally from the medium and increase its chemical potential, as quantitatively analyzed according to Figure 3a.

State I in Figure 3a is the initial equilibrium state in which the chemical potentials of water and F-actin in the F-actin solutions are μ_w^i and μ_f, respectively. The osmotic stress P_f applied to the F-actin solution moves the solution from state I to III irreversibly, changing the chemical potential of F-actin by $\Delta\mu_f$, and the chemical potential of water in the outside buffered bath by $-\bar{V}_w P_f$. To evaluate the irreversible process along I → III thermodynamically, we assume an alternative process in which the solution reaches state III along two reversible paths of I → R and R → III, where R represents a theoretically defined state in which the chemical potential of water in the F-actin solution is equilibrated with that in the outside buffered bath in the presence of osmotic stress P_f.

The I → R is the path along which the F-actin solution changes the chemical potentials of water and F-actin by $\Delta\mu_w^i = -\bar{V}_w P_f$ and $\Delta\mu_f$, respectively, with the help of the minimum work W_1, while maintaining equilibrium in itself. In

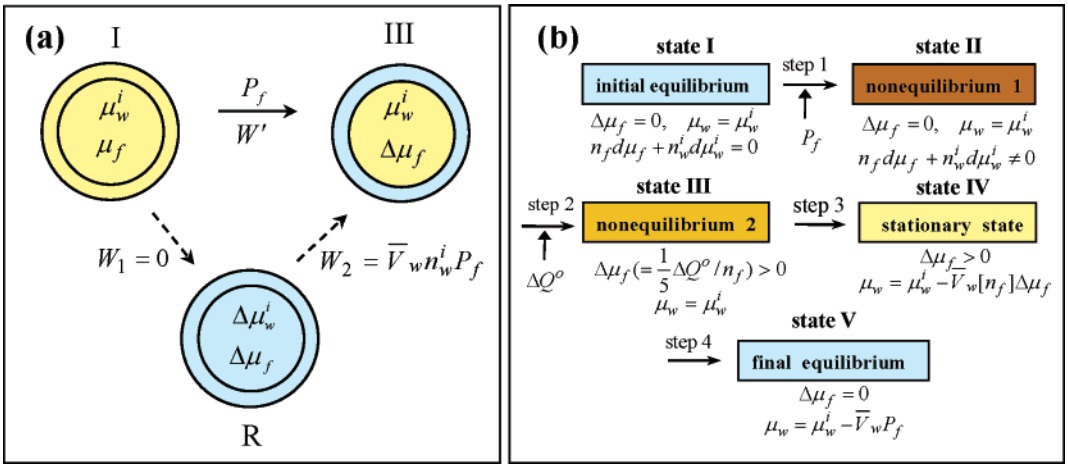

Figure 3. (a) A diagram of the thermodynamic states assumed by the F-actin solution in the presence of osmotic stress. I is the initial equilibrium state in the absence of osmotic stress in which the chemical potentials of water and F-actin in the F-actin solution are μ_w^i and μ_f, respectively, and III is the nonequilibrium state in the presence of osmotic stress P_f, in which the chemical potential of water and a change in the chemical potential of F-actin are μ_w^i and $\Delta\mu_f$, respectively. *R* is a theoretically defined state in the presence of osmotic stress P_f, in which the chemical potential of water in the F-actin solution is equilibrated with that in the outside buffered bath. Along I → R, the F-actin solution changes the chemical potentials of water and F-actin by $\Delta\mu_w^i = -\bar{V}_w P_f$ and $\Delta\mu_f$, respectively, with the help of the minimum work W_1, maintaining equilibrium in itself. R → III is the reversible path along which the F-actin solution reversibly increases the chemical potential of water from $\mu_w^i - \bar{V}_w P_f$to μ_w^i with the help of the minimum work W_2. W' is the work done on the F-actin solution along I → III by the osmotic stress P_f. (b) The proposed scheme of the entire process activated by osmotic stress is shown. μ_w is the chemical potential of water in the F-actin solution in each state denoted above, and $\Delta\mu_f$ is the deviation of the chemical potential of F-actin from its equilibrium value. P_f and ΔQ^o are osmotic stress applied to the system and heat absorbed from the medium, respectively. See the text for details.

this case, the Gibbs−Dhuem relation represented by $n_w^i \Delta\mu_w^i + n_f \Delta\mu_f - V^i \Delta P^i = 0$ holds in the F-actin solution, where $\Delta\mu_w^i$ corresponds to the total change of the chemical potential that includes the change caused by the pressure change $\bar{V}_w \Delta P^i$. Hence, the following relation holds:

$$\Delta\mu_f = -\frac{n_w^i}{n_f}\Delta\mu_w^i + \frac{V^i}{n_f}\Delta P^i \leq -\frac{n_w^i}{n_f}\Delta\mu_w^i = \frac{P_f}{[n_f]} \qquad (28)$$

since the relation $\Delta P^i \leq 0$ holds along the path.[14] According to eq 9, the minimum work W_1 to bring the F-actin solution to state R should be zero, because $W_1 = (\mu_w^i - \mu_w^o)n_w^i = 0$ at R where $\mu_w^i = \mu_w^o$.[15]

The R → III is the reversible path along which the F-actin solution reversibly changes its state with the help of the minimum work W_2, increasing the chemical potential of water from $\mu_w^i - \bar{V}_w P_f$ to $\mu_w{}^i$ without changing the chemical potential of F-actin μ_f. In this case, $W_2 = n_w^i(\mu_w^i - \mu_w^o) = n_w^i[\mu_w^i - (\mu_w^i - \bar{V}_w P_f)] = \bar{V}_w n_w^i P_f$.

Thus, the total minimum work, $W_1 + W_2$, along the path of I → R → III is equal to W_2. This work corresponds to the least amount of work to shift the F-actin solution from state I to III, because W_2 is the minimum work to create the osmotic imbalance, P_f. Hence, the work done by the osmotic stress W' should be larger than W_2:

$$W' \geq W_2 = \bar{V}_w n_w^i P_f \qquad (29)$$

From eq 28, $\Delta\mu_f \leq P_f/[n_f]$, that is, the chemical potential of F-actin is changeable up to $P_f/[n_f]$, when the F-actin solution is subjected to the osmotic stress P_f. As expected, this inequality is consistent with that in eq 25 that is derived from the law of increase of entropy.

Next, we discuss how the F-actin increases its chemical potential along the path of I → III. The osmotic stress can be assumed to be created adiabatically in this process. In this case, the following equation holds for the work done by osmotic stress W', according to the law of energy conservation:

$$\Delta Q^i = -W' - \Delta Q^o \qquad (30)$$

where ΔQ^i and $-\Delta Q^o$ are the heat absorbed by the F-actin solution (i.e., the body) and the heat removed from the outside buffer bath (the medium), respectively. Applying the first law to the body gives

$$\Delta Q^i = -W' + \Delta E^i + P\Delta V^i = -W' + \Delta(E^i + PV^i) = -W' + \Delta H^i \qquad (31)$$

where ΔH^i is the enthalpy change of the body. Combining eqs 30−31 with eq 27 gives

$$n_f \Delta\mu_f = -\frac{1}{5}\Delta Q^o \qquad (32)$$

Thus, *F-actin increases its chemical potential along the path of I → III by absorption of heat from the medium, which is mediated by the work done by osmotic stress.*

The observed value of $\epsilon = 0.24$ at 2 mg/mL F-actin indicates that the quantity of heat absorbed by F-actin, ΔQ^o, estimated by eq 25 and eq 32, is 7×10^{-20} J/single subunit at P_f = 20 cm·H_2O, and it should be linearly proportional to P_f.

2.6. Entire Process Activated by Osmotic Stress. Figure 3b shows a plausible scheme of the entire process activated by the osmotic stress. Pressure P^i is constant at the individual states from I to IV in this scheme. In equilibrium state I, the F-actin solution is in equilibrium with the outside buffer bath. Destruction of the equilibrium by osmotic stress P_f shifts the system to nonequilibrium state II. The osmotic stress does not change any thermodynamic quantity in this step, but the Gibss−Dhuem relation under constant pressure, that is, $n_f\, d\mu_f + n_w^i\, d\mu_w^i = 0$ no longer holds in this nonequilibrium state. Thus, the chemical potential of F-actin is changeable independently of a change in the chemical potential of water.

In step 2, the F-actin solution goes to nonequilibrium state III, absorbing ΔQ^o from the medium with the help of W' as discussed above, and increasing its chemical potential according to eq 32. In this step, there is no change in the chemical potential of water.

Then in step 3, the solution approaches stationary state IV, in which the solution itself is equilibrated, but not with the external environment; that is, $X \equiv -\partial S^t/\partial x = (1/T)\Delta\mu_w \neq 0$, $Y \equiv -\partial S^t/\partial y = (1/T)n_f[1 + n_w^i/n_f(d\mu_w^i/d\mu_f)] = 0$, as discussed in section 2.3. The equilibrium condition, $Y = 0$, gives the same relation between $\Delta\mu_w^i$ and $\Delta\mu_f$ as expressed by eq 24, that is, $n_w^i\Delta\mu_w^i + n_f\Delta\mu_f = 0$. Thus the chemical potential of water in the F-actin solution μ_w in this state is evaluated as

$$\mu_w = \mu_w^i - \bar{V}_w[n_f]\Delta\mu_f \tag{33}$$

where μ_w^i is the chemical potential of water in state I. It should be noted that there is no water flow from the F-actin solution until state IV.

Finally, the solution proceeds to final equilibrium state V (i.e., $X = 0$, $Y = 0$) in step 4, accompanied by water flow to the outside buffer bath, which is driven by the chemical potential difference between the two compartments, that is, $(\Delta\mu_w)_{Y=0} = \bar{V}_w(P_f - [n_f]\Delta\mu_f)$, as expressed by eq 25.

To check the rationality of this proposed process, entropy production Δ_iS in the F-actin solution at each step is evaluated. The condition $\Delta_iS > 0$ is necessary to allow each step to progress. The inequalities of $P_f > [n_f]\Delta\mu_f$ given by eq 25 or eq 29 and $W' \geq W_2$ by eq 29 directly provide the conditions for $\Delta_iS > 0$ in the change from state I to III via II:

$$T\Delta_iS = T\Delta S^i - \Delta Q^i = W' - (\Delta E^i + P\Delta V^i - T\Delta S^i) = W' - \Delta(H^i - TS^i) = W' - n_f\Delta\mu_f > W_2 - n_f\Delta\mu_f = \bar{V}_w n_w^i(P_f - [n_f]\Delta\mu_f) > 0 \tag{34}$$

where ΔS^i and ΔE^i are changes in the entropy and energy of the F-actin solution, respectively. Therefore, an increase in the chemical potential of F-actin less than $P_f/[n_f]$ may occur along with the osmotic stress-induced transition from state I to III via II.

In step 3, the F-actin solution in nonequilibrium state III changes to stationary state IV. As discussed above, the equation of $n_w^i\Delta\mu_w^i + n_f\Delta\mu_f = 0$ holds in state IV. Thus, the entropy production in this step is

$$T\Delta_iS = T\Delta S^i - \Delta Q^i = T\Delta S^i - \Delta H^i = -n_w^i\Delta\mu_w^i = n_f\Delta\mu_f > 0 \tag{35}$$

Therefore, the process in step 3 proceeds spontaneously, and the chemical potential of water reaches a stationary value of $\mu_w^i - \bar{V}_w[n_f]\Delta\mu_f$ in state IV. In the next step 4, the entire system approaches complete equilibrium, accompanied by water flow from the F-actin solution.

2.7. Effects of Actin Filament Cross-Linking on Water Flow.

If water flow in step 4 in Figure 3b is thermodynamically unfavorable, no water flow should occur, that is, $\epsilon = 1$ in eq 12. This might be the case in F-actin networks cross-linked by filamin A. In those networks, water flow stopped completely in the setting of the same osmotic stress that only slowed the flow in an un-cross-linked F-actin solution at the same actin concentration >0.5 mg/mL, as shown in Figure 4a.[9]

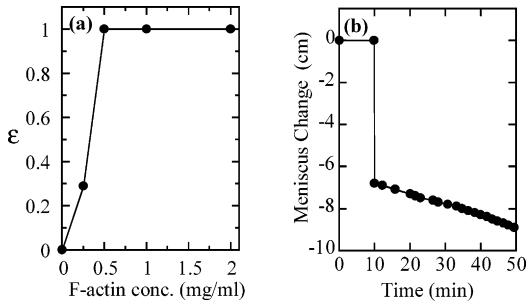

Figure 4. (a) Concentration dependence of ϵ of F-actin networks cross-linked by filamin A with a molar ratio to actin of 1:340. (From the data of Ito et al.[9]) (b) An abrupt change in the meniscus observed in the 2 mg/mL F-actin network at an osmotic stress of 40 cm·H_2O is shown. The F-actin network was subjected to osmotic stress at $t = 0$.

Cross-linking of actin filaments by filamin A immobilizes the individual filaments in solution. Consequently, if water flow occurred, it should separate a part of the network from aqueous phase. In this case, some of the filaments would be exposed to air, while the others would remain in the aqueous phase. However, such water flow from the network is thermodynamically impossible, because the entropy production resulting from a decrease in the amount of water in the network is negative, mainly because of the contribution of additional surface energy of F-actin in the phase exposed to air, as analyzed below.

Entropy production Δ_iS accompanied by water flow can be represented by

$$\Delta_iS = -(1/T)(\Delta\mu_w\Delta n_w^i + \Delta\mu_f^*\Delta n_f^*) = -(1/T)(\Delta\mu_w - \bar{V}_w[n_f]\Delta\mu_f^*)\Delta n_w^i \tag{36}$$

where Δn_f^* is the number of the filaments exposed to air, which is caused by a decrease in the amount of water in the network, $-\Delta n_w^i$, and $\Delta\mu_f^* = \mu_f^* - \mu_f$, where μ_f^* and μ_f are the chemical potentials of the filaments exposed to air and in the aqueous phase, respectively. A necessary condition for water flow from the network is $\Delta_iS > 0$. Combining this with eq 22 gives the condition for the water flow:

$$\Delta\mu_f^* < \Delta\mu_w/\bar{V}_w[n_f] < P_f/[n_f] \tag{37}$$

We shall estimate the numerical values of both sides of the inequality 37 under the conditions of $P_f = 20$ cm·H_2O and 2 mg/mL actin filaments with a length of 1 μm. The value for $P_f/[n_f]$ on the right side is calculated directly to be $\sim 2.4 \times 10^{-17}$ J/single filament. The value for $\Delta\mu_f^*$ on the left side is evaluated as follows. The surface of the actin filament is hydrophilic. Thus, in the part of the network exposed to air, the filament surface must be covered with a thin water tube with a radius approximately the same as the filaments, that is, $\sim$4 nm. In this case, the surface tension of the water tube adds to the filaments an additional energy of $\sim 2.4 \times 10^{-15}$ J/single filament, $\sim$100 times larger than that of $P_f/[n_f]$, which is calculated using 7×10^{-2} N/m as the tension coefficient of water. Hence, the inequality in eq 37 does not hold in the F-actin network used in the experiments. Consequently, no water flow from the network can be induced by the osmotic stress, as shown in Figure 4a. In this case, the network should hold the entire amount of water under the osmotic stress, fulfilling the condition that $X = (1/T)\Delta\mu_w = 0$ and $Y = (1/T)n_f[1 + n_w^i/n_f(d\mu_w^i/d\mu_f)] = 0$. This is attainable by counterbalancing the osmotic stress with a change in the chemical potential of the F-actin in the network, that is, $P_f = [n_f]\Delta\mu_f$, which is derived from $\epsilon = 1$ in eq 25.

It is worth noting that, in this case, a massive amount of energy is stored in the network structure under moderate conditions. Under the above conditions of $P_f = 20$ cm·H_2O and 2 mg/mL actin filaments, the energy stored per single actin subunit in the network is 6×10^{-20} J. This is nearly equivalent to the energy stored in the high energy bond of ATP. So, the network structure will be broken if the intensity of osmotic stress exceeds a critical value. In fact, in a 2 mg/mL F-actin network cross-linked by filamin A with a molar ratio to actin subunit of 1:340, an emergent water flow with an "infinite" rate was observed at an osmotic stress of 40 cm·H_2O, as shown in Figure 4b, presumably resulting from the collapse of the network structure by the osmotic stress.

3. Discussion

3.1. Effects of Friction and Dextran in F-Actin Solution. One may suspect the possibility that the observed inhibitory effect of F-actin on the osmotic stress-induced water flow is due to frictional properties of F-actin. As analyzed in section 2.1, frictional force on the solvent molecule in the F-actin solution is less than 0.03% of that in the osmometer membrane (eq 4). Or, in the case of the filamin A cross-linked F-actin gel that forms a perpendicularly cross-linked network and stops water flow completely (Figure 4a), the frictional force can be evaluated directly. A mesh diameter of the 2 mg/mL actin gel is approximately 0.5 μm, whereas the mesh diameter of the osmometer membrane with a nominal cutoff molecular weight of 20 kD is 3 nm. In this case, the frictional force on the water molecule in the actin gel is less than 0.004% of that in the membrane, assuming that the frictional force is inversely proportional to the area of the mesh pore. Hence, flow of water in the F-actin solution or gel is nearly free of friction, compared with that in the membrane. Thus, we cannot attribute the hindrance of the osmotically driven water flow by F-acin to any frictional force.

A high molecular weight additive such as poly(ethylene glycol) is reported to change the physical state of the assembly structure of F-actin in solution.[16] The F-actin solutions used in the experiments contained 20 mg/mL dextran with an average molecular weight of 40 kD, which may affect osmotic stress-induced water flow by changing the assembly structure of F-actin in the solution and/or the physical properties of the solution. Therefore, the effects of the dextran on the assembly structure and viscoelastic properties of the F-actin solution were investigated by static light scattering and oscillating rheometry, respectively. The presence of dextran did not change the static light scattering or storage/loss modulus of the F-actin solution significantly. The increase in light scattering at 450 nm of 2 mg/mL F-actin solution was less than 1% upon addition of dextran, and the storage and loss moduli of the solution at a frequency of 5 rad/sec were $\sim$3.0 and $\sim$3.5 N/m^2, respectively, with or without dextran. These results strongly indicate that the dextran in solution does not interact with the actin filaments in a way to change their assembly structure or physical properties such as viscoelasticity.

3.2. The Le Chatelier's Principle-Governed F-Actin Response to Osmotic Stress. The second law of thermodynamics-based Le Chatelier's principle, which states "an external interaction that disturbs the equilibrium brings about processes in the body which tend to reduce the effects of this interaction", is recognized well as the response of thermodynamic system to various interactions that disturb equilibrium. In this report, we have shown that the Le Chatelier's principle governs such a response of actin filaments to osmotic stress as shown in Figure 2. To demonstrate more directly how the Le Chatelier's principle works on this system, we shall compare two alternative paths for the F-actin solution to reach a stationary state. One is the path that leads to the stationary state with $\Delta\mu_f > 0$ and $\mu_w = \mu_w^i - \bar{V}_w[n_f]\Delta\mu_f$ (state IV in Figure 3b), and the other leads to the stationary state with $\Delta\mu_f = 0$ and $\mu_w = \mu_w^i$. Even though the free energy change along each path is the same, the law of entropy dictates that the former path is more favorable, as discussed in section 2.3. This leads to a stationary state with a smaller difference in chemical potential of water between the inside and outside of the F-actin solution, resulting in a reduction of disturbance applied by the osmotic stress, as is consistent with the prediction of the Le Chatelier's principle.

To our knowledge, this is the first report demonstrating that the Le Chatelier's principle applied to the response of biopolymers to an external disturbance such as osmotic stress. To elucidate the mechanism, we have addressed the novel problem of how the osmotic stress changes the chemical potential of F-actin (Figure 3). The osmotic stress imposed across a semipermeable membrane may help F-actin to absorb heat from an external medium isothermally, resulting in an increase in the chemical potential (see eqs 27, 32). This effect of osmotic stress has not been reported previously, presumably because of the absence of experimental setup enabling quantitative analysis for osmotic stress-induced water flow both theoretically and experimentally as done here.

3.3. Reaction Coupled with Isothermal Heat Absorption. As described above, the increase in chemical potential of a solute mediated by osmotic stress is due to isothermal heat absorption of the solute from the outside medium. From a theoretical view, only a reaction with an activation energy of the same order of or less than the thermal energy kT can couple with isothermal heat absorption. Consequently, a reaction accompanied by a conformation change in a protein, which involves an activation energy larger than kT, is not expected to be induced isothermally under osmotic stress. Thus, a monomeric protein such as G-actin cannot respond to osmotic stress. In addition, a polymer with rubber elasticity such as dextran cannot respond to osmotic stress, because the internal energy of the polymer depends only on temperature, so that isothermal heat absorption of the trigger for the response to osmotic stress does not occur in step 2 in Figure 3b.

Garcia et al. detected fluorescence spectra changes in pyrenyl emission of N−(1-prenyl)iodoacetamide-labeled F-actin in response to a large hydrostatic pressure of $\sim$200 atm.[17] This spectra change may be due to a conformation change of the subunit molecules. In contrast, no spectra change occurred in N−(1-prenyl)iodoacetamide-labeled F-actin, when the solution in a dialysis bag was subjected to osmotic stress of up to 400 cm·H_2O (data not shown), as expected from the above discussion.

The increase in the chemical potential of F-actin under osmotic stress may be due to a thermally activated change in subunit−subunit interaction, which is not accompanied by the change in the subunit conformation. According to an analysis of the variations in crossover spacing in actin filaments observed in electron micrographs by Egelmann et al.,[18,19] a random deviation of $5 \sim 6°$ (rms) exists in the angular position of the nearest neighbors of each subunit of actin filament. This deviation may result from thermal fluctuation of the position of the individual subunit molecules in the filament, which could endow semiflexible properties to the filament.[20] The existence of the fluctuation indicates that the activation energy for the change in the angular position is smaller than kT. As a

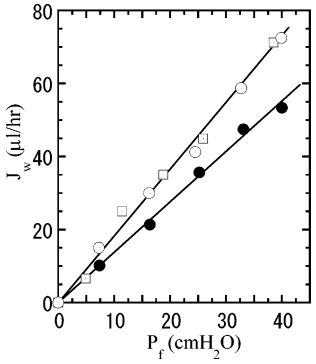

Figure 5. The effects of glutaraldehyde treatment of actin filaments on the osmotic stress-induced water flow. For the glutaraldehyde treatment, 2 mL of a 2.0 mg/mL F-actin solution in the osmometer vessel was incubated with 300 mL of the F-buffer (100 mM KCl, 2 mM $MgCl_2$, 0.2 mM $CaCl_2$, 1 mM ATP, 1 mM DTT, 10 mM imidazole chloride, pH 7.5) containing 5% glutaraldehyde for 1 day at 4 °C. The osmotic stress-induced water flow J_w was measured as described previously. The values of J_w are plotted against various intensities of the osmotic stress P_f. Shown are (●) 2.0 mg/mL F-actin solution, (○) 2.0 mg/mL F-actin solution treated with glutaraldehyde, and (□) a control solution without F-actin.

consequence, the individual subunit molecule may vary its angular position through isothermal heat absorption and modulate subunit−subunit interaction to increase the internal energy of the filament as much as the law of entropy requires.

To confirm the above prediction, we have investigated the effects of glutaraldehyde-treament of action filaments on the osmotic stress-induced water flow (Figure 5). The closed circles, open circles, and open squares in Figure 5 represent osmotic stress-induced water flows of 2.0 mg/mL F-actin, 2.0 mg/mL F-actin treated by 5% glutaraldehyde, and a control solution without F-actin, respectively. The nontreated F-actin solution retarded water flow with an ϵ value of 0.24 (●), while the glutaraldehyde-treated F-actin solution did not retard it at all (○). We may interpret these results according to the discussion described above. The intermolecular cross-linking between the subunits by glutaraldehyde may fix the position of each subunit in the filament, making the activation energy for a position change much larger than kT, so that any change in the subunit−subunit interaction through isothermal heat absorption could not occur to increase the internal energy of the filaments in response to osmotic stress. As the result, there is no retardation of osmotic stress-induced water flow in the glutaraldehyde-treated F-actin solution.

3.4. Profile of Entropy Change in Stationary State. The experiments reported here are the measurements of water flow in step 4 in Figure 3b, where the entire system in the stationary state approaches the final equilibrium state, accompanied with a change in entropy as discussed in section 2.1. Those entropy changes in the individual F-actin systems are summarized in Figure 6, where we represent the entropy of the system as a deviation from that in the final equilibrium state ΔS^t (see Figure 2b), plotting $-\Delta S^t$ as a function of the chemical potential difference of water between the inside and outside of the osmometer $\Delta\mu_w$, and the chemical potential increase of F-actin $\Delta\mu_f$.

In the absence of F-actin, the system approaches the final equilibrium state (point o in Figure 6) along the yellow line A on which $\Delta\mu_w = \bar{V}_w P_f$ and $\Delta\mu_f = 0$. In the presence of F-actin, the system approaches to the equilibrium along the blue line B on which $\Delta\mu_w = \bar{V}_w(1 - \epsilon)P_f$ (point a) and $\Delta\mu_f = \epsilon P_f/[n_f]$ (point b), retarding osmotic stress-induced water flow that is proportional to $\Delta\mu_w$.

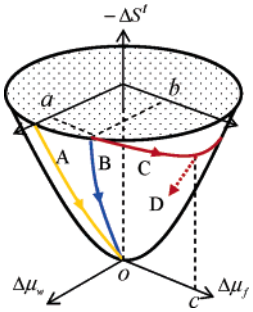

Figure 6. The change in negative entropy difference, $-\Delta S^t$, in the stationary state as a function of the difference in chemical potential of water between the inside of the osmometer and outside buffer bath, $\Delta\mu_w$, and the chemical potential increase of F-actin, $\Delta\mu_f$. The continuous lines of A, B, and C represent the changes in $-\Delta S^t$ in the absence of F-actin, in the presence of F-actin, and in the presence of filamin A cross-linked F-actin, respectively. Points a and b on the $\Delta\mu_w$ and $\Delta\mu_f$ axes in an equi-entropy plane (dotted plain) correspond to $\Delta\mu_w = \bar{V}_w(1 - \epsilon)P_f$ and $\Delta\mu_f = \epsilon P_f/[n_f]$, respectively, and point c to $\Delta\mu_w = 0$, $\Delta\mu_f = P_f/[n_f]$. Dotted arrow D represents collapse of the gel caused by the osmotic stress above the threshold intensity.

In the case of the F-actin network cross-linked by filamin A, the entire system reaches the metastable state at point c of $\Delta\mu_w = 0$ and $\Delta\mu_f = P_f/[n_f]$, where $-\Delta S^t$ reaches another minimum, and no water flow occurs. Above the critical intensity of osmotic stress, the network collapses accompanied by a transient "infinite" water flow shown by an abrupt change in the meniscus of the osmometer capillary (Figure 4b),[21] and the system finally reaches the stable state of point o (dotted red line D).

3.4. Biopolymer Networks. Cross-linked polymer networks respond to osmotic stress to equilibrate an osmotic imbalance between the inside and outside of the network. In a polymer network with rubber elasticity, a change in *the entropy* of the network strands dominates the equilibration, so that a large change in the volume of the networks occurs in response to osmotic stress.[5−7] In contrast, in a network of F-actin filaments, a change in the energy of the network strands dominates the equilibration, as discussed in this report, so that equilibration can be established without any significant change in the volume of the network (Figure 4a). This response to osmotic stress may be characteristic of biopolymer networks constructed of protein subunits. In fact, networks of an intermediate filament of vimentin responded to osmotic stress in a manner similar to F-actin (data not shown). This type of response of biopolymer networks has certain physiological implications. Mammalian cells may be constantly subjected to fluctuations in osmotic pressure that cause cell swelling or shrinkage, and the biopolymer networks in the periphery of the cells could constitute a relatively strong buffer against such fluctuations to prevent significant changes in cell volume.

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(12) The pressure-dependent term of the chemical potential is $\bar{V}_w P^i$. Thus, $d_i\mu_w^i$ [$d_i\mu_w^i = d(\mu_w^{i\prime} - \bar{V}_w P^i)$] corresponds to the pressure-independent term in the total differential.

(13) Equation 24 is derived from eq 23 under the assumption that n_w^i is constant. This assumption is reasonable because the deviation of n_w^i from its equilibrium value is negligibly small (less than 5%) in the present experiments.

(14) The relation $\Delta P^i \leq 0$ may be due to the positive osmotic stress P_f (≥ 0) applied to the system in the path I → R. The positive osmotic stress should induce water flow from the inside to outside across the membrane and thus decrease the hydrostatic pressure on the solution.

(15) The relation $W_1 = n_w^i(\mu_w^i - \mu_w^o)$ is derived from eq 9, which is a homogeneous function of the first order in n_w^i in the case of $n_w^i\, d_i\mu_w^i + n_f\, d_i\mu_f = 0$ (Gibbs−Dhuem relation).

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(21) The collapse of the network should be accompanied by "infinite" water flow in a manner as follows. The collapse of the network abruptly increases the chemical potential of water in the network to change $\Delta\mu_w$ from 0 to $\bar{V}_w(1 - \epsilon)P_f$. As a consequence, a transient 'infinite' gradient of the chemical potential of water appears at the boundary between the inside of the osmometer and semipermeable membrane, and abrupt induction of the "infinite" water flow occurs at this moment, because the water flow is proportional to the chemical potential gradient (see the discussion described above eq 2 in section 2.1).