

# The Hydration of Actin

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**The hydration of actin was studied by differential scanning calorimetry between  $-30^{\circ}\text{C}$  and  $30^{\circ}\text{C}$  and by thermogravimetric analysis. The differential scanning calorimetry provided the freezable water content of G- and F-actin as a function of concentration, and the thermogravimetric analysis measured the total water content. The difference between the two yielded the nonfreezable water content (bound water) as a function of concentration. The nonfreezable water content of G-actin was higher than the F-actin over the whole concentration range from 1–40% actin. Invest Ophthalmol Vis Sci 32:562–566, 1991**

Actin is present in plant and mammalian cells as either G-actin (globular, water-soluble, monomeric form) or F-actin (filamentous, polymerized form).<sup>1</sup> The two forms are interconvertible. G-actin, the globular monomer with 5-nm diameter, polymerizes in high ionic strength buffers to form a double helix with a half pitch of 36–38 nm.<sup>2</sup> F-actin forms the thin or microfilaments of the cytoskeletal bodies, and it is assumed to influence the shape of the cells. Actin occurs also in the lens fiber cells of different vertebrate species including humans.<sup>3</sup> It is particularly concentrated at the short side of the hexagonal lens fiber cells, along the length of the lens fiber plasma membrane. Actin is associated with plasma membrane in both cortical and nuclear fiber cells of calf and chicken lenses.<sup>4</sup> It is influential in the differentiation of the fiber cells and is associated with the more spheric accommodated state of the lens.<sup>5</sup>

In providing transparency to the lens, the theory requires that the alignment of birefringent cytoskeletal bodies balance the form birefringence produced by the plasma membranes of fiber cell organization. In this manner the orientation fluctuation (or optical anisotropy fluctuation) is minimized, providing maximum transparency.<sup>6,7</sup> Any process that upsets this balance, plasma membrane disintegration, and/or cytoskeletal body disorientation therefore will contribute to turbidity or cataract formation.<sup>8</sup> To elucidate the role of the different cytoskeletal bodies in transparency–cataract relationships, we must know the optical and hydrational properties of the constitu-

ent molecules. We describe the hydration properties of G- and F-actins.

## Materials and Methods

G-actin was isolated from fresh chicken gizzards by the methods of Strzelecka-Golaszewska et al.<sup>9</sup> It was further purified by gel permeation chromatography using a Sephadex G-100 column (2" diameter  $\times$  14" height, Pharmacia LKB, Biotechnology Inc., Piscataway, NJ) equilibrated with buffer containing 0.1 mM adenosine triphosphate (ATP), 0.2 mM  $\text{CaCl}_2$ , 2 mM Tris HCl, and 0.005 mM  $\text{NaN}_3$ , pH 7.6–8.0. G-actin was eluted at a flow rate of 30 ml/hr at  $4^{\circ}\text{C}$ . Fractions of 10 ml were collected and analyzed for absorbance at 280 nm. The main fraction, ie, G-actin, was then polymerized at  $23^{\circ}\text{C}$  by adding ATP, KCl, and  $\text{MgCl}_2$  sequentially to make a final concentration of 0.2 mM, 30 mM, and 1 mM, respectively. F-actin was collected by centrifugation at 20,000 g for 1 hr at  $4^{\circ}\text{C}$ . Other samples of G-actin collected from the gel permeation chromatography were lyophilized for further study.

Three different samples were prepared for the hydration study: (1) G-actin diluted with distilled water buffered at pH 7.1; (2) F-actin diluted with distilled water buffered at pH 7.1; and (3) F-actin diluted with the high ionic strength polymerization medium, containing KCl,  $\text{MgCl}_2$ , and ATP. The solutions of actin were prepared from the lyophilized samples by adding the proper media. The concentration of the actin solutions was determined by thermogravimetric analysis.

Approximately 10-mg samples of G- and F-actins with different concentrations were placed in aluminum pans. The sample weight was obtained by subtracting the tare from the weight of the sealed aluminum pan containing the samples. The samples were stored at  $-30^{\circ}\text{C}$  until the thermal properties were measured. For analysis of the freezable water content,

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differential scanning calorimetry (DSC) was used. A hermetically sealed, empty, coated aluminum pan served as the reference. The sample and reference pans were placed in a DSC (DuPont 990; Dupont, Wilmington, DE) cell and cooled to  $-30^{\circ}\text{C}$  by an external dry ice-acetone bath. The DSC curves were obtained by heating the sample at a programmed rate of  $3^{\circ}\text{C}/\text{min}$ . Most experiments were done in a  $\text{N}_2$  atmosphere with a flow rate of  $50 \text{ mm}^3/\text{min}$ . The instrument was calibrated with a sapphire disc, and the DSC cell calibration constant was obtained periodically. The DSC curves recorded the differential heat flow ( $dq$ ) as a function of time. The  $dq$  was recorded simultaneously with two different sensitivities, for example,  $0.5 \text{ mV}/\text{cm}$  (high sensitivity) and  $10 \text{ mV}/\text{cm}$  (low sensitivity).

The area under the curve gives the number of joules of heat used to melt the measured mass of water. Since it was our intention to convert the area of an endotherm (in joules per gram of sample) into a certain amount of freezable water per gram of sample, we ran a calibration curve with distilled water and with aqueous NaCl solutions with different concentrations having different melting (freezing) points.<sup>10</sup>

After the DSC measurements, the pans were punctured, taking care not to disturb the samples. The pans were next placed in a thermogravimetric analyzer (DuPont 951), and the total water content of the samples was obtained from the weight loss which occurred during heating the pans to and maintaining them at  $105^{\circ}\text{C}$ . The nonfreezable water content was

obtained as the difference between the total and freezable water content, expressed as a percentage of the total water content or as milligrams of water per gram dry weight.

## Results

The freezable water content of actin obtained from the DSC measurements can be identified with the free water content. This is presented in two graphic forms: (1) as grams of freezable water per gram of actin as a function of actin concentration (Fig. 1) and (2) as freezable water percent of the total water content as a function of actin concentration (Fig. 2). The statistical significance of the data can be ascertained from the following considerations. To get the freezable water content in grams per gram of sample, the experimentally obtained value in joules per gram of sample was multiplied by the instrument constant obtained from the calibration curve, ie,  $351.2 \pm 1.4 \text{ J/g}$  freezable water. This average value was obtained on a large number of samples. The error  $\pm 1.4 \text{ J/g}$  was equivalent to  $\pm 0.4\%$ , and it includes the random and systemic errors in weighing the sample, in differential heat flow measurements, and in computer integration of the areas. Thus, the error in our measurement of freezable water content was  $\pm 0.4\%$ . The error in thermogravimetric measurement was  $\pm 0.25\%$ . The similarity between the two errors was due to the fact that weighing the samples was the least precise measurement, and this occurs in both calculations. Rather than inserting too many error bars, we can appreciate the reproducibility of the results by apply-

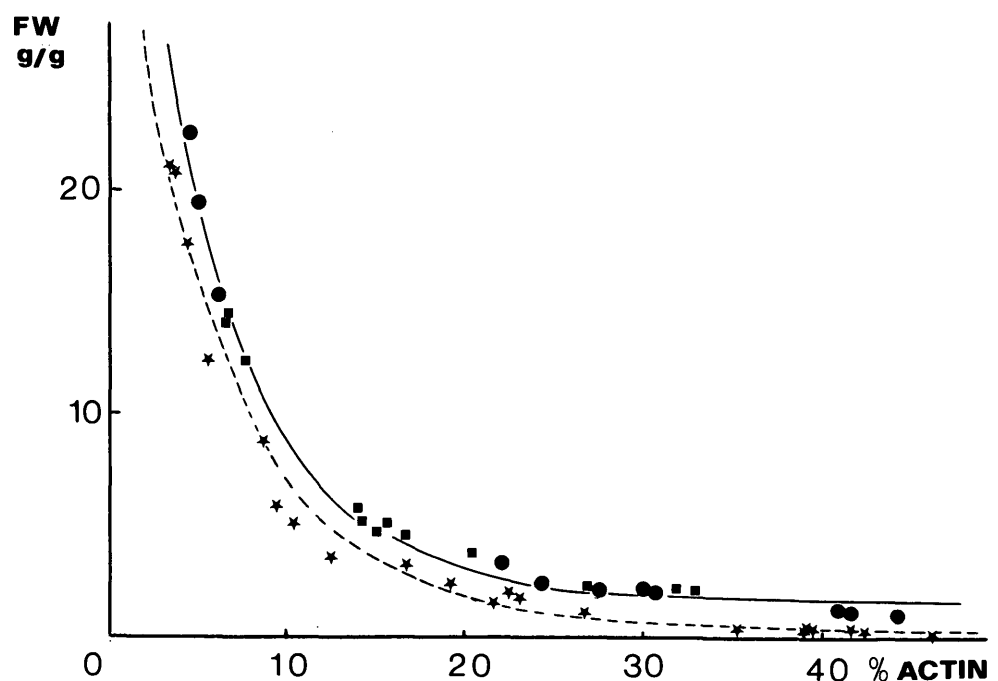


Fig. 1. Freezable water content of actin (g water/g actin) as a function of actin concentration.  $\star$ — $\star$  G-actin;  $\bullet$ — $\bullet$  F-actin in water;  $\blacksquare$ — $\blacksquare$  F-actin in high ionic strength polymerizing medium.

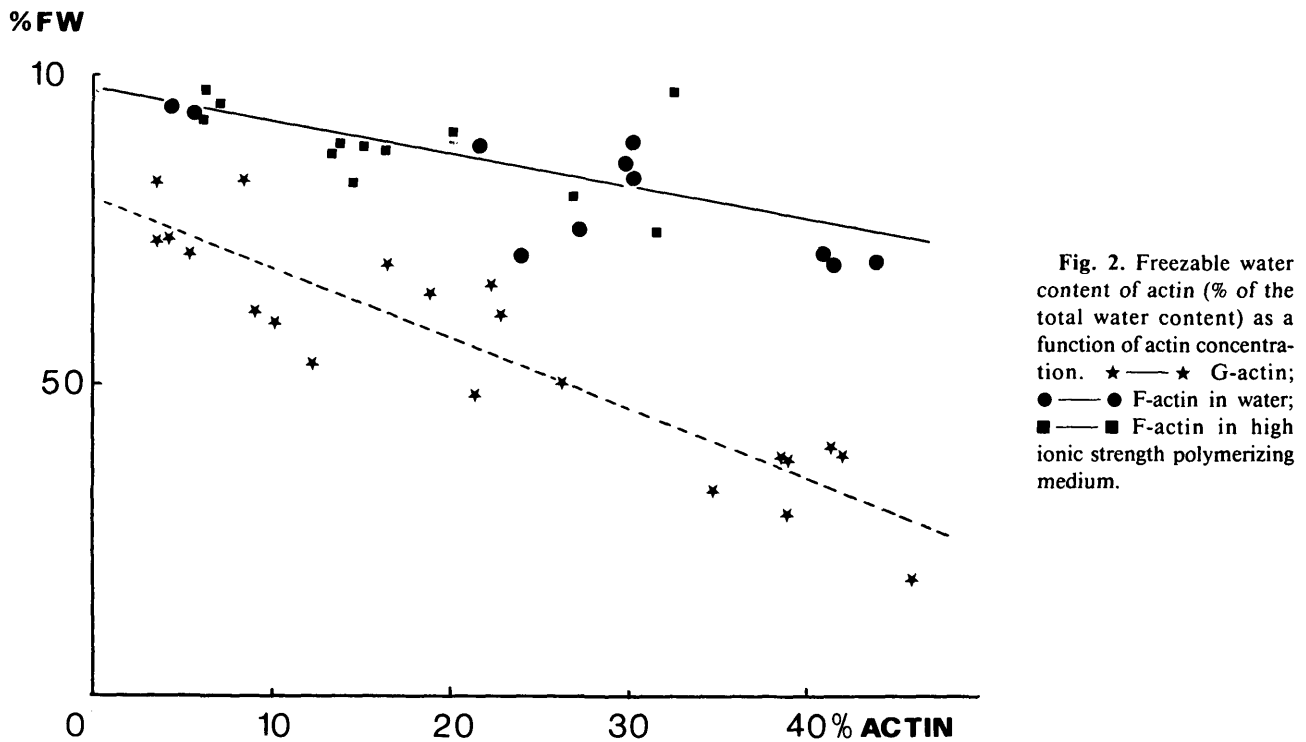


Fig. 2. Freezable water content of actin (% of the total water content) as a function of actin concentration. ★ — ★ G-actin; ● — ● F-actin in water; ■ — ■ F-actin in high ionic strength polymerizing medium.

ing this  $\pm 0.4\%$  to each and every freezable water content measurement. This in essence is the accuracy which involves both random and systematic errors; the reproducibility, ie, the precision of the measurement involves only random errors which are the major contributor of the error. In both cases (Figs. 1, 2) the diagrams show an inverse relationship, ie, as the actin concentration increases, the free water content decreases. The difference between G- and F-actin hydration behavior is clear in both presentations. The G-actin has a lower freezable water content than the F-actin at all actin concentrations. This difference was greater when the water content is presented as the percent of the total water (Fig. 2) rather than as the grams of water per grams of actin (Fig. 1). The hydration of F-actin did not seem to be influenced by the environment. Within experimental error, it was the same whether the imbibing liquid was distilled water or a polymerization solution of high ionic strength. The fitting of the freezable water data (Fig. 2) to a straight line had  $r^2 = 0.86$  as a correlation coefficient, and the standard deviation was 6.56 residual for G-actin. Similar data for F-actin were  $r^2 = 0.82$  and standard deviation = 4.99.

The nonfreezable water content can be obtained from the difference between the total water content of the thermogravimetric analyzer (TGA) measurements and the freezable water content of the DSC experiments. This water content can be identified as bound water. In Figure 3 the nonfreezable water con-

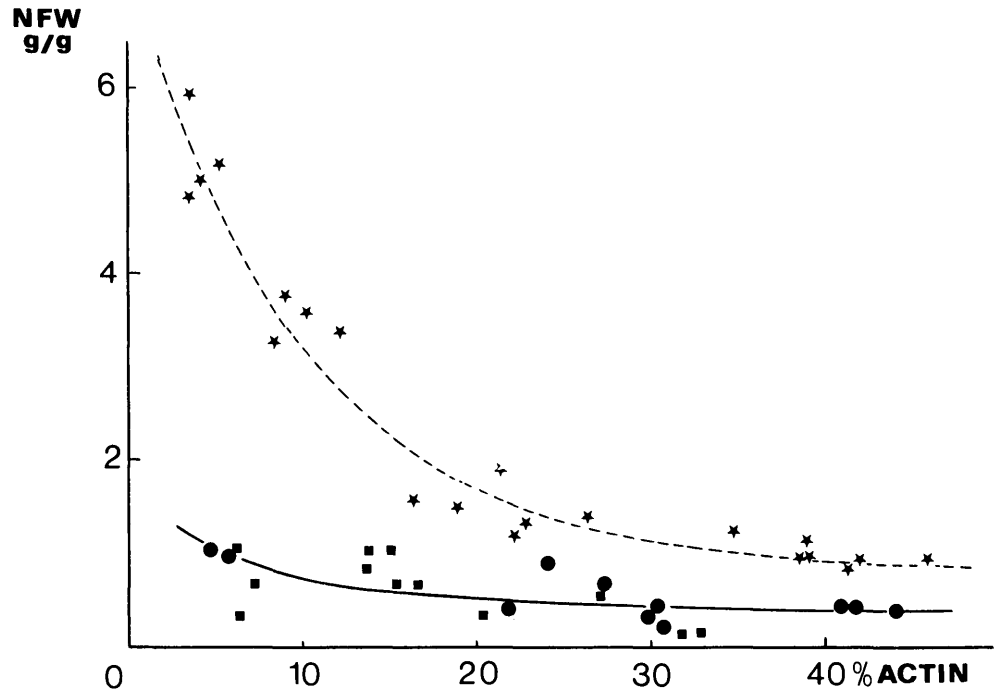
tent is given as grams of nonfreezable water per gram of actin as a function of actin concentration. Again the relationship was inverse. The nonfreezable water content decreased with actin concentration. This decrease was very steep at low F-actin concentrations, and it leveled off after about 10% actin concentration. Again there was no significant difference in the hydration behavior of F-actin, whether the swelling medium was distilled water or the polymerization solution. The G-actin showed significantly greater bound (nonfreezable) water content than did F-actin.

This was even more evident when the nonfreezable water content was calculated as the percent of the total water content (Fig. 4). In this presentation the nonfreezable water content increased with actin concentration. This occurred because as the actin concentration increased, the total water content decreased to a greater extent than the nonfreezable water, and thus the proportion of the nonfreezable water as a part of the total water increased. The same hydration behavior was observed; G-actin had more bound water than F-actin.

### Discussion

The most important result of this study was that the nonfreezable water contents of G-actin and F-actin differ at identical actin concentrations. G-actin contains more nonfreezable water than F-actin irrespective of whether we calculate it as grams of non-

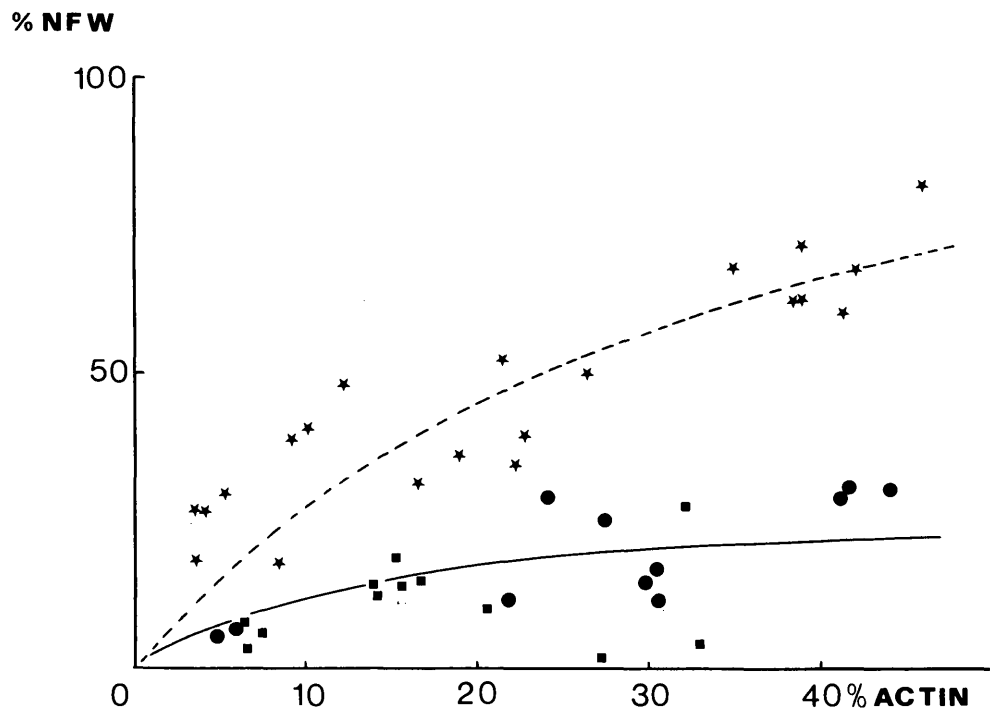
**Fig. 3.** Nonfreezable water content of actin (g water/g actin) as a function of actin concentration.   
 ★ — ★ G-actin; ● — ● F-actin in water; ■ — ■ F-actin in high ionic strength polymerizing medium.



freezable water per gram of actin or as the percent of nonfreezable water of the total water content. Thus, a hypothetical transition from G- to F-actin involves the loss of nonfreezable water content at any actin concentration. This may have physiologic significance since it has been shown<sup>11</sup> that the elongation of the lens epithelial cells is associated with a shift from G- to F-actin.

To evaluate the significance of our result, we must consider the nature of nonfreezable water. Its most common interpretation is that this portion of water in the vicinity of physical and/or macromolecular surfaces is already fully or partially immobilized (bound) and partially organized so that there is no phase transition to ice on cooling or to liquid (bulk) water on melting.<sup>12,13</sup> An alternative explanation is based on

**Fig. 4.** Nonfreezable water content of actin (% of total water content) as a function of actin concentration.   
 ★ — ★ G-actin; ● — ● F-actin in water; ■ — ■ F-actin in high ionic strength polymerizing medium.



kinetic arguments. According to this, as the temperature of a solution is lowered, the solution becomes freeze concentrated, and its viscosity increases. Thus supersaturation occurs which allows crystallization only over infinitely long periods. In this interpretation, the nonfreezable water cannot be identified with a special kind of (bound) water, and its content would depend on the boundary conditions of the experiment.<sup>13</sup> Even if we accept the "bound" water interpretation of the nonfreezable water content, there is still a problem: different measurements show different nonfreezable water contents under the same boundary conditions. Different modes of nuclear magnetic resonance analysis yield lower nonfreezable water content than the combined DSC-TGA thermal analysis, although always showing the same trend among different substances.<sup>14</sup>

Our data imply that a transition from G- to F-actin releases some water from the "bound" state into the bulk. Such a process is equivalent to syneresis.<sup>15</sup> We showed that a syneretic process is operative in the change of the amplitude of density fluctuations of the crystallins in the lens of the eye which leads to cataract formation.<sup>8</sup> The current study was initiated to identify the nature of hydration of actin, a major cytoskeletal body in the lens of the eye. The actin of smooth muscles (chicken gizzard) and of nonmuscle cells (lens) are of the same type ( $\beta$  and  $\gamma$ ).<sup>5</sup> Originally we thought that actin would influence transparency/turbidity, and hence cataract formation, only through its orientation fluctuation in the fiber cell. Thus a higher organization or more parallel alignment or disorganization would upset the equilibrium between form and intrinsic birefringence which, in the normal lens, balance each other, leading to a very small total birefringence.<sup>6,7</sup> This in turn enhances transparency. This study shows, however, that transition from G- to F-actin and vice versa can also influence density fluctuation through a syneretic process. Thus, the cytoskeletal bodies may contribute to transparency and turbidity in both the density and orientation fluctuations.

Beyond this, the loss of bound water on polymerization of G- to F-actin may imply that the overall conformation and inner hydration of G-actin may remain the same after polymerization. Only the actual physical surface in contact with the environment

is reduced on polymerization and that affects the "bound" water content. This interpretation is enhanced by the findings that the hydration properties of F-actin do not depend on the nature of the medium, whether it is distilled water or aqueous polymerization medium.

**Key words:** actin, freezable water, differential scanning calorimetry, nonfreezable water, thermogravimetric analysis

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