コイG-アクチンの重合におよぼすCa2+の効果

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The Effect of Ca^{2+} on the Polymerization of Carp G-Actin

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The effect of Ca^{2+} on the G–F transformation of carp G-actin was examined by both the precipitation method and the ultracentrifugation method. The transformation started at a Ca^{2+} concentration of 0.06 mmol/(g of G-actin) and was completed at the concentration of 0.30 mmol/(g of G-actin). This means that 10 mol of Ca^{2+} is necessary for 1 mol G-actin to polymerize to F-actin. Such a transformation process can be considered to be the nucleation of F-actin, where the G-actin exists as dimer or trimer. Then, the F-actin nuclei may polymerize to form F-actin molecules.

It is a well-known fact that G-actin molecules polymerize to form a long filament of F-actin and that this polymerization is promoted very much by the binding of divalent cations to G-actin molecules. Ca^{2+} plays an especially important role in this polymerization.

Many studies on actin since 1942 by Straub[1] have been made mainly on samples from rabbit muscle. However, few authors[2–4] have reported on fish actin.

Recently, Strzelecka-Glaszewska, *et al.*[5] have reported the results of their studies on the effect of divalent cations on the G–F transformation of rabbit muscle actin. They have found that the G-actin molecule has a single high-affinity site, and five low-affinity sites for divalent cations. We think it is important to obtain such information about fish proteins from the food technological point of view, too. In this work, we have studied the effect of Ca^{2+} on the polymerization of G-actin from carp muscle.

Experimental

Preparation of Actin

Acetone dried muscle powder was prepared from the muscle of carp, *Cyprinus carpio*, using the procedure described by Arai, *et al.*[6] Actin was extracted for 40 min at 0~2°C with a 2 mmol tris-HCl buffer containing 0.5mmol ATP and 0.2 mmol $CaCl_2$ from the acetone powder, and purified by the Spudich and Watt method.[7] The purity was ascertained by SDS-polyacrylamide gel electrophoresis, ultracentrifugation, and a viscosity measurement. These methods are described in detail in the following paragraph.

Characterization of Actin

(1) SDS-Polyacrylamide gel electrophoresis. The SDS-polyacrylamide gel electrophoresis was done by the Weber-Osborn procedure.[8] The electrophoresis was performed at a constant current of 8 mA per gel. The proteins used as the references were obtained from Boehringer Mannheim: cytochrom c (molecular weight, M=12,500), chymotrypsinogen A (M=25,000), ovalbumin (M=45,000), and bovine serum albumin (M=67,000).

(2) Ultracentrifugation. The sedimentation equilibrium experiments were performed with a Hitachi Model UCA-1 ultracentrifuge using interference optics. The rotor speed was set at 11,200 rpm based on the molecular weight and the partial specific volume reported. The temperature was maintained at 20°C. All runs were made at that speed for approximately 24 h so as to ensure that an equilibrium was established in a liquid column of 1.5 mm. The sedimentation velocity experiments were performed with the same ultracentrifuge as was used in the sedimentation equilibrium experiments, however, Schlieren optics were used instead of the interference optics in these experiments. The rotor speed was set at 21,410~59,000 rpm, and the temperature was maintained at 25°C.

(3) Viscosity measurement. The viscosity measurements were made with an Ostwald type viscometer with outflow time of approximately

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100 s for water at 15°C.

Determination of the Yield of F-Actin

Ca^{2+} were added to G-actin solutions by dialysis; G-actin in a 2 mmol tris-HCl buffer solution containing ATP (0.5 mmol/*l*) at pH 7.6 were dialized against Ca^{2+} solution ($CaCl_2$ solution) for 24 h at 20°C. The dializing time, 24 h, was experimentally chosen as described in the results section of this paper. After dialysis for 24 h at 20°C, the solution was centrifuged at 100,000×*g* for 3 h, and the protein concentration of the supernatant was quantitatively measured by a modified LOWRY technique.[9] Then the quantity of the precipitate, which is the polymer of G-actin, *i. e.* F-actin, was estimated.

Results and Discussion

The Molecular Parameters of the G-Actin

The sedimentation velocity pattern of the G-actin solution before the addition of Ca^{2+} showed a sharp single peak. The concentration dependence of sedimentation coefficients is shown in Fig. 1. From the plot in Fig. 1, the sedimentation constant, $s^0_{20,w}$, was determined to be 3.12 S (Svedberg unit), which agreed with the value of 3.02 S reported by KAY[10] on rabbit G-actin.

The SDS-polyacrylamide gel electrophoresis pattern of the sample showed one band, from which the molecular weight of the G-actin was determined to be 43,000. The molecular weight of 43,000 agrees with the reported values on rabbit G-actin by several other researchers.[11]

The data from the sedimentation equilibrium measurements are shown in Fig. 2. Namely, logarithms of f are plotted against the square of distance, r^2, where f is the displacement of a fringe, this corresponds to the concentration difference. Because the plot has a curvature near the cell bottom as shown in Fig. 2, the G-actin is considered to be polymerized in the higher concentration region. The molecular weight of 42,000 was determined from the plot in the lower concentration region near the meniscus.

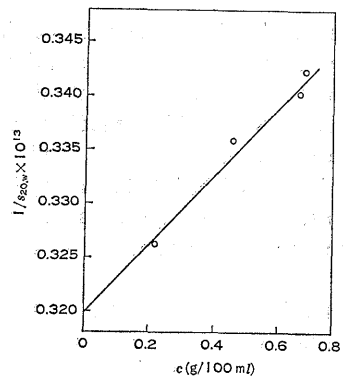

Fig. 1. The concentration dependence of sedimentation coefficient of G-actin.

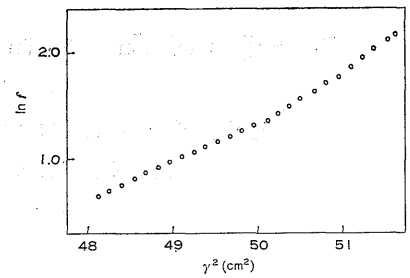

Fig. 2. Plots of logarithm of f versus the square of distance. f corresponds to the concentration difference.

Fig. 3 shows the time dependence of the viscosity of the actin solution after the addition of KCl. As shown in Fig. 3, the viscosity of the actin solution increased with the addition of 0.1 mol/*l* KCl. The viscosity, increasing with time, attained a stationary value after 90 min. From this result, the actin used in this study was ascertained to have an ability to polymerize.[12]

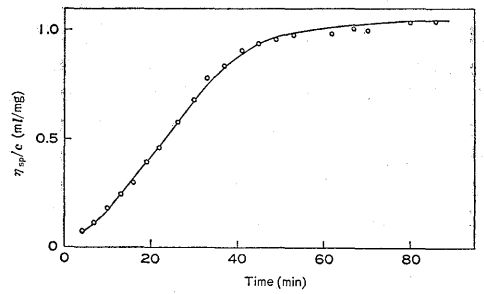

Fig. 3. The time dependence of the viscosity of the actin solution after the addition of KCl.

The Effect of Ca^{2+} on the Formation of F-Actin

The dializing time was decided from the measurement of precipitation with the G-actin solution, of which concentration was 2.80 mg/m*l*, containing Ca^{2+} of 1.5 mmol/*l*. The quantity (%) of the precipitate, *i. e.* F-actin, is plotted against the dializing time in Fig. 4. From Fig. 4, it is clear that the quantity of precipitate formed attains a constant value after 18 h. On the basis of this result, all the dialysis were made for 24 h so as to ensure that

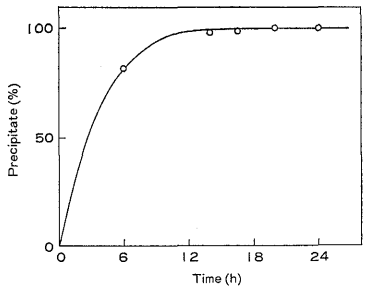

Fig. 4. Plots of the quantity (%) of the precipitate (F-actin) versus the dializing time.

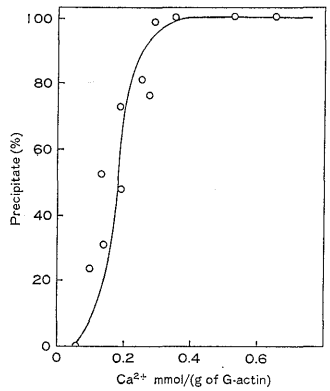

Fig. 5. Plots of the quantity (%) of the precipitate (F-actin) versus Ca^{2+} concentration.

the F-transformation of G-actin was complete.

Fig. 5 shows the effect of Ca^{2+} on the G–F transformation of actin. The concentration of G-actin used was *ca.* 3.0 mg/m*l*, and the concentration range of Ca^{2+} was from 0.2 mmol/*l* to 2.0 mmol/*l*. In this figure, the ordinate is the quantity (%) of precipitate, *i. e.* F-actin, and the abscissa, the concentration of Ca^{2+} (mmol/g of G-actin). The transformation occurred abruptly within the narrow range of the Ca^{2+} concentration. As shown in Fig. 5, G-actin began to polymerize with a Ca^{2+} concentration of over 0.06 mmol/(g of G-actin), and the polymerization was complete at the Ca^{2+} concentration of 0.30 mmol/(g of G-actin). Thus, the Ca^{2+} concentration which can essentially contribute to the transformation of all the G-actin to F-actin can be estimated to be 0.24 mmol/(g of G-actin). The sedimentation velocity pattern of the solution containing Ca^{2+} of 0.20 mmol/(g of G-actin), which was in the transition region of the transformation, showed two peaks and a gel component. Plate 1 shows the sedimentation pattern of the actin solution containing 0.02 mmol/(g of G-actin) of Ca^{2+}. The protein

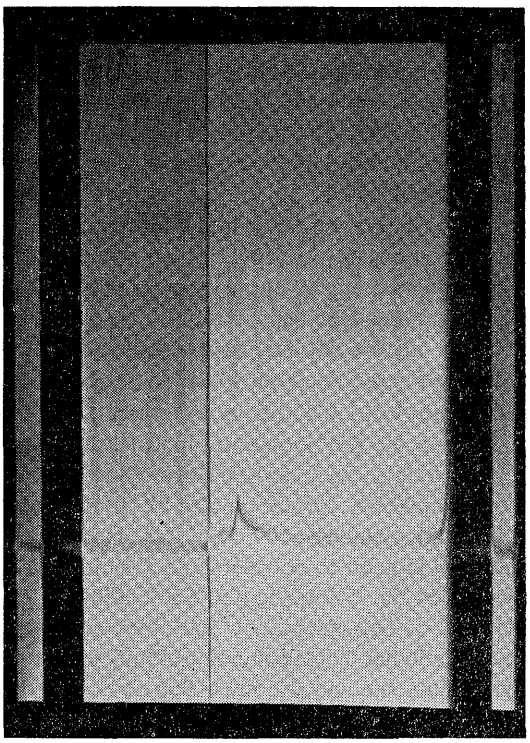

Plate 1. The sedimentation pattern of the actin solution containing 0.20 mmol/(g of G-actin) of Ca^{2+}. The protein concentration was 3.22 mg/m*l*. The photograph was taken at 10 min after attainment of full speed, 21, 410 rpm.

concentration was 3.22 mg/m*l*. The photograph was taken at 10 min after attainment of the full speed, 21,410 rpm, 20°C. The sedimentation coefficients of 30 S and 4 S are estimated from the rapid sedimenting peak and from the slow sedimenting peak, respectively. The gel component could not be shown in the photograph, since it sedimented rapidly during the accelating time.

OOSAWA, *et al.*[13] have stated that G-actin polymerizes over a definite concentration which they call the critical concentration, and have suggested that there is the step of nucleation in the initial stage of polymerization. Moreover, the molecular weight of F-actin was reported to be 8.0×10^6 on the basis of the light scattering data.[14] The 30 S component is considered to be F-actin molecules which have the molecular weight of 8.0×10^6, although the molecule should be assumed to have less flexibility from the relationship between the molecular weight of 8.0×10^6 and the sedimentation coefficient of 30 S. The gel component detected in this experiment may correspond to the F-actin filament. MARUYAMA, *et al.*[15] have found

that F-actin has a particle size distribution, and have suggested that the distribution comes from either the association-dissociation of F-actin or the association-dissociation between F-actin and G-actin. However, the sedimentation pattern in Plate 1 shows only the two narrow components. As described above, on the assumption that the gel component corresponds to the F-actin filament, the gel component probably has the size distribution as pointed out by MARUYAMA, *et al.* On the other hand, the 4 S component must be considered to be the mixture of oligomer and monomer of G-actin in the process as the so-called nucleation.[18]

Using the value of 43,000 as the molecular weight of G-actin, the Ca^{2+} concentration of 0.24 mmol/(g of G-actin), which can transform G-actin to F-actin completely, corresponds to be 10 mol/(mol of G-actin). Namely, 10 mol of Ca^{2+} is necessary for 1 mol G-actin to polymerize to F-actin, and it means that the G-actin has 10 Ca^{2+} affinity sites. STRZELECKA-GLASZEWSKA, *et al.*[5] have suggested that G-actin has 6 divalent cation affinity sites, one high affinity site and five low-affinity sites, on the results of their gel-filtration method using radioactive divalent cations. The 10 sites evaluated in this work appear to roughly support their suggestion, although the difference of affinity could not be ascertained.

When the transformation mechanism is assumed to be a kind of adsorption reaction of Ca^{2+} to G-actin molecule, the mechanism is similar to the LANGMUIR's adsorption isotherm from the shape of the curve in Fig. 5. On this assumption, this means that the G-actin molecule has a definite number of adsorption sites, which can hold one adsorbed Ca^{2+} ion site by site, and that there is no remarkable interaction between Ca^{2+} ions on different sites.

Equilibrium Constant and the Degrees of Polymerization

The equilibrium constant, K_n, of the reaction

$$n\mathrm{M} \rightleftharpoons \mathrm{M}_n,$$

where M is a monomer and M_n the n-mer, can be expressed as follows:

$$K_n=[\mathrm{M}_n]/[\mathrm{M}]^n,$$

where $[M_n]$ and [M] are the concentrations of n-mer and monomer, respectively.

GORDON, *et al.*[16] obtained the value of −36.4 kJ/mol for the GIBBS' free energy change, ΔG°, of the Ca^{2+} induced polymerization of G-actin from the viscosity measurement. On the other hand, ENGEL, *et al.*[17] have reported that the value of ΔG° was in the range from −39 to −59 kJ/mol at pH 8 and 25°C on the basis of the rate constant of the steady state propagation from the ATP-G-actin to ADP-F-actin. Using the well-known relationships,

$$\Delta G^\circ=-RT\ln K_n,$$

where R is the gas constant and T the absolute temperature, the equilibrium constant, K_n, is estimated to be $9\times10^6\sim3\times10^{10}$ $(l/\mathrm{mol})^{n-1}$ from the ΔG° values of $-39\sim-59$ kJ/mol. Therefore, the degree of polymerization, n, is evaluated to be *ca.* 3. As described before, the trimer is considered to be the nuclei of F-actin. On the assumption that the value of ΔG° linearly changes with the logarithm of Ca^{2+} concentration in the transition region, we can calculate the equilibrium constants and then the n-values at arbitrary Ca^{2+} concentrations. The results are shown in Table 1. From these results, it is concluded that the G–F transformation starts at the Ca^{2+} concentration of 0.06 mmol/(g of G-actin) and is completed at that of 0.30 mmol/(g of G-actin), and that the transformation process can be considered to be the so-called nucleation. Then after that, the F-actin molecule may be formed by polymerizing the F-actin nuclei. Finally the F-actin molecules may aggregate to form long filaments. The other experiments to ascertain the nuclei, *i. e.* the oligomer of G-actin, are now being planned.

Table 1. The Gibbs' free energy change and the equilibrium constant of the Ca^{2+} induced polymerization of G-actin, and the degree of polymerization

Ca^{2+}	ΔG°	K	n
0.06	0	0	—
0.10	−12 ~ −19	$1.55\times10^2\sim1.26\times10^3$	2
0.15	−22 ~ −34	$9.08\times10^3\sim9.78\times10^5$	2
0.20	−29 ~ −44	$1.61\times10^5\sim7.28\times10^7$	2
0.25	−35 ~ −52	$1.47\times10^6\sim2.11\times10^9$	3

Ca^{2+}: g of G-actin, ΔG°: kJ/mol,
K: $(l/\mathrm{mol})^{n-1}$.

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