NOTE

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Evaluation of Diffusion Coefficients of Myosins from Sedimentation Boundary Curve

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Fujita[1,2] showed that the height-area ratios of schlieren patterns obtained during a sedimentation velocity experiment can be used for the evaluation of the diffusion coefficient of the solute component in solution. This indicates that sedimentation and diffusion coefficients can be obtained under the same conditions. Considering the labile and restrictive nature of purified biological active macromolecules available for physico-chemical measurements, the evaluation of molecular weight from sedimentation and diffusion coefficients is still valuable.

The reported molecular weights of skeletal myosin are 4.2×10^5—6.2×10^5. The discrepancies were discussed in terms of the presence of aggregates in the samples.[3,4] In the present study, the diffusion coefficients for myosins prepared from smooth and skeletal muscles are evaluated from sedimentation boundary curves using Fujita's method, and the molecular weights of these myosins are calculated from the sedimentation and diffusion coefficients extrapolated to infinite dilution.

Experimental

Preparation of Sample. Smooth muscle myosin (myosin-S) was prepared from parts of the smooth muscle of horse gullet by ammonium sulfate fractionation.[5,6] Skeletal muscle myosin (myosin-A) prepared from rabbit skeletal muscle by the ammonium sulfate fractionation method[7] was supplied by Prof. T. Sekine. These myosins were dissolved in 0.5 M KCl solution containing 1/150 M phosphate buffer (pH 7.5). All sedimentation measurements were performed within three days after the final purification to minimize the formation of aggregates in the sample solutions. Concentrations of the solutions were determined from the optical densities at 279 nm. The extinction coefficients used were 600 cm^2/g[6] for myosin-S and 560 cm^2/g[8] for myosin-A.

Ultracentrifugal Experiment. A Hitachi UCA-1 ultracentrifuge equipped with schlieren optics was used. The temperature of the rotor was kept at 5.0 ± 0.1 °C. A synthetic boundary cell of double sector type and a conventional cell were used. To avoid direct contact of the sample solution with a metallic surface, which sometimes causes denaturation of proteins, either a cell made of epoxy resin or an aluminum cell coated with Teflon was used. The speed of the rotor was 10490—60000 rpm.

Results and Discussion

Sedimentation Coefficient. Sedimentation coefficients were calculated from the rates of movement of the boundary peak at several rotor speeds. The following equation[9] was used to correct the sedimentation coefficients at higher rotor speeds for pressure effect:

$$\ln(r_*/r_a)/\omega^2 t = S^0(C_0)\{1+[k_sC_0-m(1+2k_sC_0)/2(1+k_sC_0)][(r_*/r_a)^2-1]\} \quad (1)$$

where r_* and r_a are the distances from the center of rotation to the peak and the solution meniscus, respectively, ω is angular velocity, t time, $S^0(C_0)$ the sedimentation coefficient at concentration C_0, which is the initial concentration of the solution, m a parameter, and k_s a positive parameter characteristic of a given system. For the data at rotor speeds lower than 32000 rpm, at which the peak sedimented slowly and the boundary diffused quickly, the following equation was used.[10]

$$-2S\omega^2 t = \ln\left\{1-\left(\int_{r_a}^{r_p}(r^2-r_a^2)(dc/dr)dr\right)\Big/C_0r_a^2\right\} \quad (2)$$

where r_p is the distance from the center of rotation to a point arbitrarily chosen in the plateau region. The values of S obtained by Eq. (2) were equated to $S^0(C_0)$. Values of t were corrected graphically by using the conditions where $\ln(r_*/r_a)$ vanishes at $t=0$. In all experiments, the coefficient for the term $[(r_*/r_a)^2-1]$ in Eq. (1) was very small, no significant dependence of sedimentation coefficient on angular velocity being found. The sedimentation coefficients were corrected for concentration dependence by

$$1/S^0(C_0) = (1/S_0)(1+k_sC_0) \quad (3)$$

Figure 1 shows the concentration dependence of the sedimentation coefficient for myosin-S, the data being expressed by $1/S^0(C_0)=0.247(\pm0.001)+0.0230(\pm0.0003)C_0$ where C_0 is given in units of mg/ml. Figure 2 shows the results for myosin-A, the data being expressed by $1/S^0(C_0)=0.252(\pm0.004)+0.0246(\pm0.0003)C_0$.

Diffusion Coefficient. Data for the height-area ratio H/A from a series of sedimentation boundary curves could be used for the evaluation of diffusion

1) H. Fujita, *J. Phys. Chem.*, **63**, 1092 (1959).
2) H. Fujita, "Mathematical Theory of Sedimentation Analysis," Academic Press, New York, N. Y. (1962), p. 106.
3) W. W. Kielley, *Ann. Rev. Biochem.*, **34**, 403 (1964).
4) A. Stracher, P. P. Trotta, L. C. Gershman, and P. Dreizen, *J. Gen. Physiol.*, **50**, 85 (1967).
5) M. Yamaguchi, Y. Miyazawa, and T. Sekine, *Biochim. Biophys. Acta*, **216**, 411 (1970).
6) A. Kotera, M. Yokoyama, Y. Miyazawa, and M. Yamaguchi, *Biopolymers*, **7**, 99 (1969).
7) W. W. Kielley and L. B. Bradley, *J. Biol. Chem.*, **218**, 653 (1956).
8) W. W. Kielley, W. F. Harrington, and P. A. Small, *Biochim. Biophys. Acta*, **49**, 462 (1961).
9) Ref. (2), p. 137.
10) R. L. Baldwin, *Biochem. J.*, **55**, 644 (1953).

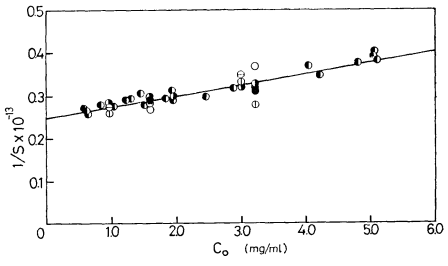

Fig. 1. Reciprocal of sedimentation coefficient for myosin-S plotted against concentration. Different symbols correspond to different rotor speeds, *i.e.*, ○: 10490 rpm; ●: 21410 rpm; ◑: 31820 rpm; ◐: 43700 rpm; ⊖: 51200 rpm; ⊕: 60000 rpm.

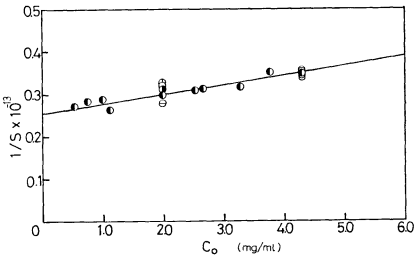

Fig. 2. Reciprocal of sedimentation coefficient for myosin-A plotted against concentration. Different symbols correspond to different rotor speeds, *i.e.*, ○: 10490 rpm; ●: 21410 rpm; ◑: 31820 rpm; ◐: 43700 rpm; ⊖: 51200 rpm; ⊕: 60000 rpm. The points at $C_0=4.34$ mg/ml read from the upper point as ◑, ●, ◐, ⊕, and ⊖.

coefficient D, when an adequate equation for H/A is available. Fujita[1,2] derived the following equation for this purpose.

$$G^{-1}(2r_a\omega^2S_0k_sC_0(H/A)t) = [r_a\omega^2k_sC_0S_0/2D^{1/2}][1-(S_0\omega^2t/2)(1-k_sC_0)]t^{1/2} \quad (4)$$

where G^{-1} is the inverse function of G. The value of D can then be determined from the slope of a plot of $[1-(1/2)(1-k_sC_0)\omega^2S_0t]t^{1/2}$ *vs.* $G^{-1}(2r_a\omega^2S_0k_sC_0(H/A)t)$. Figures 3 and 4 show the plots of $[1-(1/2)(1-k_sC_0)\omega^2S_0t]t^{1/2}$ *vs.* $G^{-1}(2r_a\omega^2S_0k_sC_0(H/A)t)$ for the smooth and skeletal muscle myosins, respectively. The zero-time corrections were made by plotting $(A/H)^2$ against time and extrapolating $(A/H)^2$ to zero. We see that the data points for each experiment lie on a straight line, most of the lines passing through the origin.

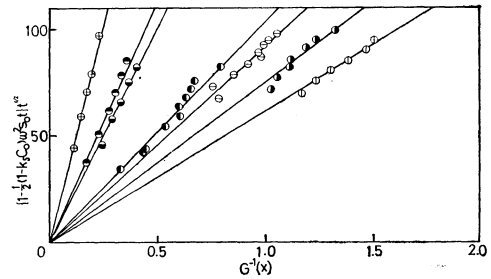

Fig. 3. Typical plots of $[1-(1/2)(1-k_sC_0)\omega^2S_0t]t^{1/2}$ against $G^{-1}(2r_g\omega^2k_sC_0S_0(H/A)t)$ for myosin-S. ⊕: $C_0=3.24$ mg/ml at 10490 rpm; ◓: 1.61 mg/ml at 21410 rpm; ◒: 0.98 mg/ml at 31820 rpm; ◐: 0.83 mg/ml; ⊖: 1.01 mg/ml; ◑: 1.25 mg/ml; ⊕: 1.95 mg/ml. The points not specified by the rotor speed were obtained at 43700 rpm.

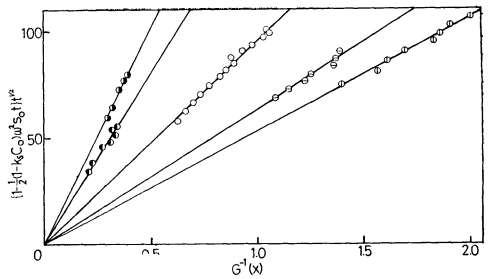

Fig. 4. Typical plots of $[1-(1/2)(1-k_sC_0)\omega^2S_0t]t^{1/2}$ against $G^{-1}(2r_a\omega^2k_sC_0S_0(H/A)t)$ for myosin-A. ◑: $C_0=2.01$ mg/ml at 21410 rpm; ◐: 0.76 mg/ml; ○: 1.13 mg/ml; ⊖: 1.85 mg/ml; ⊕: 2.46 mg/ml. The points not specified by the rotor speed were obtained at 43700 rpm.

Without zero-time corrections, however, the plots showed marked departure from the coordinate origin. According to the conditions used in deriving Eq. (4), the diffusion coefficient obtained from this equation may be taken as the one at $\bar{C}=C_0/2$.[2] The values of D for both myosins are plotted against C_0 in Fig. 5,

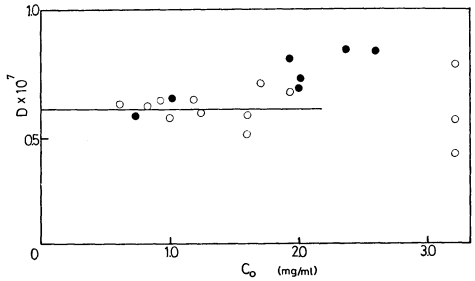

Fig. 5. Diffusion coefficients of myosins plotted against initial concentration, C_0. ○: myosin-S; ●: myosin-A.

which shows very small concentration dependence for either myosin, though the two sets of plotted points diverge considerably at higher concentrations. Considering the experimental error, the values for D at infinite dilution D_0 for both myosins may be taken as 0.64×10^{-7} cm²/sec. When this is reduced to 20 °C in water, we obtain 0.99×10^{-7} cm²/sec, which coincides with the values reported for myosin-A by other authors.[11-14] Substitution of the S_0 and D_0 values obtained into the Svedberg formula gives molecular weights of $5.2_7\times10^5$ for myosin-S and $5.1_0\times10^5$ for myosin-A. These values are slightly smaller than $5.8_7\times10^5$ for myosin-A reported by Harrington *et al.*[17] and $5.8_1\times10^5$ for myosin-S obtained from sedimantation equilibrium.[6] They are comparable with the values ranging between 5.0×10^5 and 5.5×10^5 obtained from sedimentation-diffusion measurements by others.[11-16]

11) R. G. Parrish and W. F. H. M. Mommaerts, *J. Biol. Chem.*, **209**, 901 (1954).
12) H. Mueller, J. Franzen, R. V. Rice and, R. E. Olson, *ibid.*, **239**, 1447 (1964).
13) K. Laki and W. R. Carrol, *Nature*, **175**, 389 (1955).
14) P. H. von Hippel, H. K. Schachman, P. Appel, and M. F. Morales, *Biochim. Biophys. Acta*, **28**, 504 (1958).
15) W. W. Kielley and W. F. Harrington, *ibid.*, **41**, 401 (1960).
16) P. Johson and A. J. Rowe, *Biochem. J.*, **74**, 432 (1960).
17) E. F. Woods, S. Himmelfarb, and W. F. Harrington, *J. Biol. Chem.*, **238**, 2374 (1963).