A LOW VISCOSITY G-ACTIN PREPARATION[1]

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Recently reported values for the intrinsic viscosity of G-ATP actin[2] have been about 0.1 dl/g. (e.g., Lewis et al. (1963).). This value is considerably higher than one would expect for a globular protein. The possibility remains that these preparations contain small amounts (ca. 1%) of tropomyosin, which could make a considerable contribution to the intrinsic viscosity. We wish to report some preliminary physical measurements on G-ADP actin[2] in the presence of 0.1 M salts. In addition to extracting the acetone powder at 0°C as suggested by Drabikowski and Gergely (1962), a further purification step to remove possible tropomyosin contamination was introduced.

METHODS

Acetone powder was prepared by a modification of the method of Straub (1951). The acetone powder was extracted

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[2]G-ATP actin = globular actin with ATP as its nucleotide prosthetic group. G-ADP actin = globular actin with ADP as its nucleotide prosthetic group. (Hayashi and Rosenblut (1960) and Grubhofer and Weber, (1961).

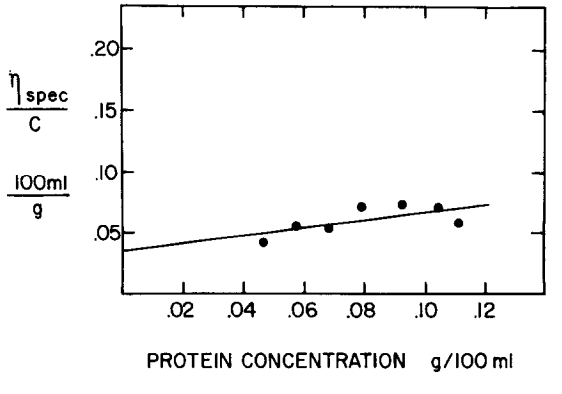
for 30 minutes with 30 volumes of water at 0°C. Purified F-actin pellets were prepared by the ultracentrifugation method of Ulbrecht et al. (1960), routinely including only two washings. G-ADP actin was prepared from the F-actin pellets as follows: F-actin was homogenized in 0.1 mM $MgCl_2$ in a glass vessel fitted with a teflon pestle for about 60 seconds. The solution was then subjected to two 30 second treatments in an MSE ultrasonicator at about 1.6 amperes. Buffer was added to give a final concentration of 10 mM, and then a 0.1 volume of 50% Dowex-1-chloride was added to remove excess nucleotide. Within a minute or two after Dowex addition the solution was filtered to remove the Dowex, and $MgCl_2$ was added to give a final concentration of 2 mM. The actin solution was then centrifuged at 105,000 g for two hours. This centrifugation should sediment any F-actin that was not depolymerized. Also, following the suggestion of Martonosi (1962) and Laki et al. (1962), at 2 mM $MgCl_2$ any tropomyosin present should sediment with the F-actin that was not depolymerized, or with any F-actin that forms because of the high $MgCl_2$ concentration. All steps in the G-ADP actin preparation were done at temperatures near 0°C to avoid polymerization which occurs at higher temperatures. Following centrifugation, the actin supernatant was diluted appropriately and salts were added to give final concentrations of 0.1 M KCl, 10 mM barbital buffer (buffer pH = 7.4 at room temperature) and 2 mM $MgCl_2$. In addition,

for the ultracentrifuge experiments salts were added to another sample to give final concentrations of 0.1 M KI, 10 mM barbital buffer (buffer pH = 7.4 at room temperature), and 2 mM $MgCl_2$. (Incubation of G-ADP actin in 0.1 M KI results in some loss of activity as judged by polymerization. For example, after 1 1/2 hours in 0.1 M KI at 0°C, polymerization at 30°C after the addition of ATP is inhibited by 35%). Viscosities were determined in Cannon-Fenske viscosimeters with solvent outflow times of 240 seconds at 0°C, and a rate of shear of approximately 850 sec^{-1}. Molecular weights were calculated from Archibald approach to equilibrium experiments assuming a value of $(1-\bar{v}p) = 0.28$. Both the viscosity and the Archibald experiments were done at 0°C. Viscosity measurements and initial molecular weight determinations were completed within 60 minutes after the addition of salt to the actin supernatant.

RESULTS

A plot of the reduced viscosity versus concentration of G-ADP actin in 0.1 M KCl at 0°C is shown in Figure 1.

Fig. 1



The line, fitted by the method of least squares, gives an intrinsic viscosity of about 0.036 dl/g. This value is in the same range as that reported for bovine serum albumin, a globular protein, and indicates that the G-ADP actin is 1 mg/ml this actin preparation polymerized at 30°C to give a reduced viscosity of about 5 dl/g at a rate of shear of approximately 1900 sec^{-1}.

Determinations of the molecular weight on the same sample in 0.1 M KCl and 0.1 M KI have been made using the Archibald approach to equilibrium method. Molecular weights determined at the meniscus early in the run were 69,500 in 0.1 M KCl and 63,500 in 0.1 M KI. These values are probably correct for this sample within ±4,000. The determinations made at the bottom of the solution column gave molecular weights under 80,000, indicating reasonable homogeneity of the preparation. These molecular weights also show that the G-ADP actin obtained from this preparation is essentially monomeric.

The ability to prepare G-ADP actin of this low viscosity has not been attained easily. Unfortunately, those conditions of pH and $MgCl_2$ concentration which facilitate obtaining the low viscosity actin, also stimulate polymerization of the higher protein concentrations at 0°C. This viscosity rise occurs at such a rate (under our conditions) that at least a few days would be required for complete polymerization. However even a 1% polymerization will give a molecular weight about 20,000 higher than the monomeric value.

The intrinsic viscosity near 0.04 dl/g brings the G-actin viscosity determinations into agreement with the shape of the molecule as anticipated from electron micrographs of Hanson and Lowy (1963). The low viscosity and the molecular weights also provide confirmation for the suggestion by Hayashi and Rosenbluth (1962) that the G-ADP actin can exist as a monomer at 0°C in the presence of salts.

REFERENCES

Drabikowski, W., and Gergely, J., (1962) J. Biol. Chem., 237 3412

Grubhofer, N., and Weber, H.H. (1961) Zeit. f. Naturforsch. 16b 435

Hanson, J., and Lowy, J., (1963) J. Mol. Biol. 6 46

Hayashi, T., and Rosenbluth, R., (1960) Biol. Bull. 119 294

Hayashi, T., and Rosenbluth, R., (1962) Biochem. Biophys. Res. Commun. 8 20

Laki, K., Maruyama, K., and Kominz, D.R. (1962) Arch. Biochem. Biophys. 98 323

Lewis, M.S., Maruyama, K., Carroll, W.R., Kominz, D.R., and Laki, K. (1963) Biochemistry 2 34

Martonosi, A., (1962) J. Biol. Chem. 237 2795

Straub, F.B., as described by A. Szent-Gyorgyi in "Chemistry of Muscular Contraction" p 148 Academic Press, New York 1951

Ulbrecht, M., Grubhofer, N., Jaisle, F., and Walter, S. (1960) Biochim. Biophys. Acta 45 443