G-ACTIN: PREPARATION BY GEL FILTRATION AND EVIDENCE FOR A DOUBLE STRANDED STRUCTURE

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Previous methods used in the preparation of G-actin (e.g. Carsten and Mommaerts, 1963 and Martonosi, 1962) entailed reversible polymerization of the protein. We have been interested in making use of Sephadex gel filtration, in order to prepare actin rapidly and in its monomeric form.

The preparation of the actin acetone powder was essentially that of Straub (1943). Following extraction with acetone and drying, the powder was extracted three times with five volumes of chloroform. The dried powder was extracted at 4°C with 5-10 volumes of glass distilled water for 45 minutes with occasional stirring. After filtration and the addition of ATP to give a concentration of 5×10^{-4} M, the supernatant was then chromatographed on a Sephadex G-200 column that had been previously equilibrated with 5×10^{-4} M ATP, pH 8.1.

Using a column 34 X 4.5 cm and applying 25 cc of actin, the elution pattern is as shown in Fig. 1. No effort has been made to identify the components of the first peak which are thought to be a mixture of tropomyosin, inactive aggregated actin and possibly some F-actin. The major peak was G-actin as demonstrated by its complete polymerization in the presence of 0.1 M KCl. Since Sephadex filtration will depolymerize actin, the assymetry in this peak is thought to be due to a small quantity of F-actin, present in the original extract which was depolymerized on chromatography.

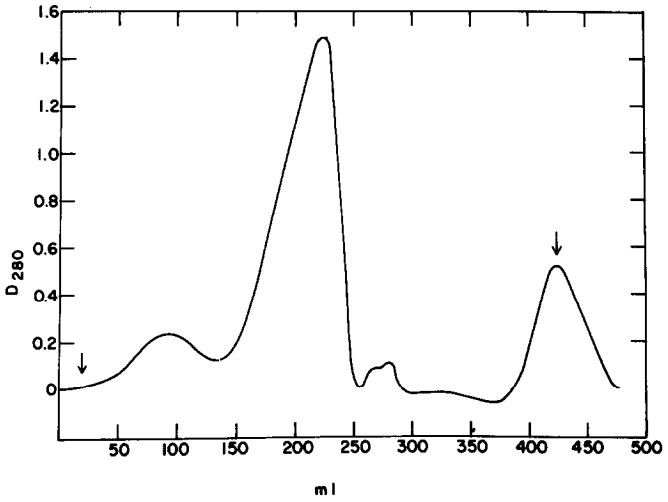

Fig. 1 Elution pattern of a crude actin extract from a Sephadex G-200 column equilibrated with 0.5 m M ATP, pH 8.1.

The third peak was negative in the biuret test and from UV difference spectra appears to be a mixture of nucleotides. The minor peak following the actin peak was not usually observed. The arrows indicate the front and the salt boundary. The abscissa does not include the void volume of 170 cc.

Re-chromatography of the actin peak on Sephadex G-200 gave a symmetrical peak. Analysis of one actin preparation for the presence of adenosinetriphosphate-creatine transphosphorylase and adenylic acid deaminase following the procedure of Kuby, Noda and Lardy (1954) and Kalckar (1947), respectively, gave negative results.

Actin, prepared in the manner described above, has a concentration of 1-2.8 mg/ml depending on the extracting ratios and column dimensions. Depending on the size of the column, the time for preparation from extraction of powder, is 10-30 hours.

We have examined the molecular weight of actin prepared in this manner by the short column sedimentation equilibrium technique. The method used was a modification of that of Richards and Schachman (1959), employing 1 mm columns and Rayleigh interference optics. Runs were made in a Spinco Model E ultracentrifuge at speeds of 15,200 r.p.m. allowing a minimum of 8 hours for the

attainment of equilibrium at 4°C. For these experiments, the actin solution contained 5 X 10^{-4} M ATP and 5 X 10^{-4} M Tris pH 7.7 and the G-200 column was equilibrated with the same solvent.

Log C vs. X^2 plots showed a slight downward curvature at the higher concentrations indicating concentration dependence. The linearity or slight downward curvature characteristic of all our observations in ATP, is an indication of the homogeneity of the actin solutions.

Fig. 2 is a graph of the reciprocal of molecular weight vs. concentration for three different preparations. C_o, the initial concentration, is expressed in the number of interference fringes, determined by a synthetic boundary run for each concentration. When the least squares analysis line is extrapolated to infinite dilution, the above plot gives a molecular weight of 47,400. The $\bar{V}$ employed in these calculations was Kay's value of 0.716. The value for the molecular weight of actin is significantly lower than the previously reported values of 56-58,000 (Mommaerts, 1952, Laki and Standeart, 1960, and Lewis et al., 1963). The value of 47,400 is probably lower than the true value for the molecular weight, because of charge effects in solutions of low ionic strength.

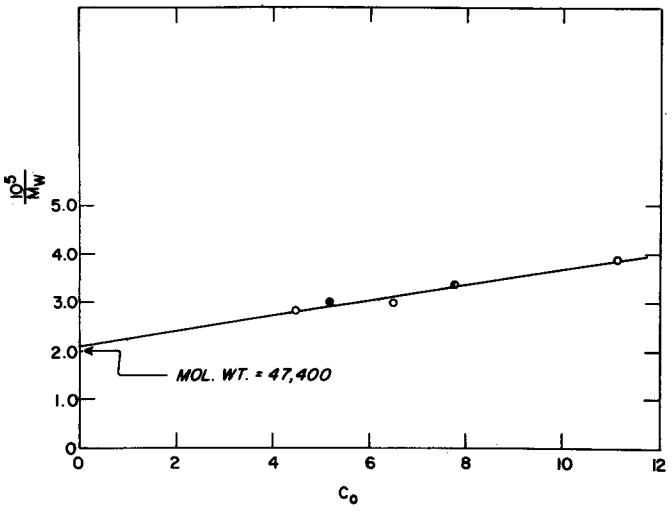

Fig. 2 Concentration dependence of the reciprocal of the molecular weight of G-actin for three different preparations. Sedimentation equilibrium run in 0.5 m M ATP and 0.5 m M Tris pH 7.7.

We have also examined the molecular weight of actin in 5 M guanidine hydrochloride using the short column sedimentation equilibrium method at 20°C. These runs were made at 10-15,000 r.p.m. allowing at least 8 hours for attainment of equilibrium. For these determinations, actin prepared in the manner outlined earlier was lyophilized, dissolved in 8 M guanidine hydrochloride and reacted with an excess of N-ethyl maleimide. The solution was then diluted to 5 M with respect to guanidine hydrochloride and dialyzed for 8-10 days against 5 M guanidine hydrochloride.

The plot of the reciprocal of molecular weight against C_o for two different preparations is seen in Fig. 3. The values used for molecular weight were obtained from the slope of the log C vs. X^2 plot. Calculation of weight average molecular weights gave essentially the same values with a deviation in the range of 1-2%. Extrapolation to infinite dilution gives a molecular weight of 28,200. This number was computed using the same $\bar{V}$ as that used in ATP runs. Observation on other proteins (Kielley and Harrington, 1960) in concentrated guanidine hydrochloride indicate a lowering of $\bar{V}$ by 1-2% in this solvent. Thus, it is anticipated that the value calculated here is of the order of 3-5% too high.

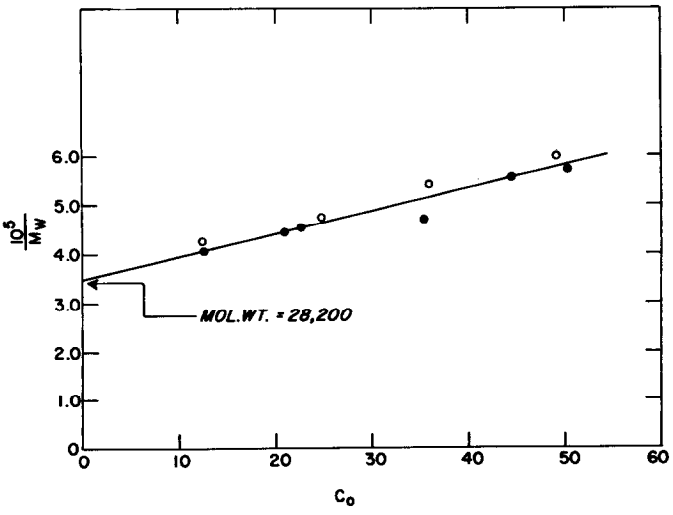

Fig. 3 Concentration dependence of the reciprocal of molecular weight of N-ethyl maleimide actin for two different preparations. Sedimentation equilibrium run in 5 M guanidine hydrochloride.

The log C vs. X^2 plots for these runs showed a slight upward curvature, suggesting some heterogeneity. Measurements of SH content of actin have indicated a lability in SH groups during preparation and storage. No special efforts to prevent loss of SH groups were employed and it is possible that this apparent heterogeneity may be due to disulfide bridges between chains, a factor which would also tend to make the observed value for the molecular weight too high. On the other hand, the apparent heterogeneity could arise from the dissociation of actin into two chains of slightly different mass. Though these preliminary observations are subject to some uncertainties, it seems quite evident that the actin molecule is composed of two polypeptide chains of similar, if not identical, mass.

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