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THE MOLECULAR WEIGHT OF ONE OF THE LARGEST KNOWN NATURAL POLYPEPTIDES: TITIN

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INTRODUCTION

Skeletal muscle titin is thought to be the largest naturally occurring polypeptide so far described. Originally isolated in denatured form (1) it has subsequently been purified as a native protein which in some extracts appears on SDS-PAGE to consist of two proteins of very similar molecular weight, titins "1" and "2" (2). Molecular weight estimations, using the relative method of SDS-PAGE have varied widely from 0.4×10^6 to "about 10^6" (1,2). In vitro, titin may aggregate to form ultra-thin filaments of diameter 6nm (2). In this short study we confirm that titin may well be the largest known polypeptide using the more reliable and absolute technique of low speed sedimentation equilibrium.

METHODS

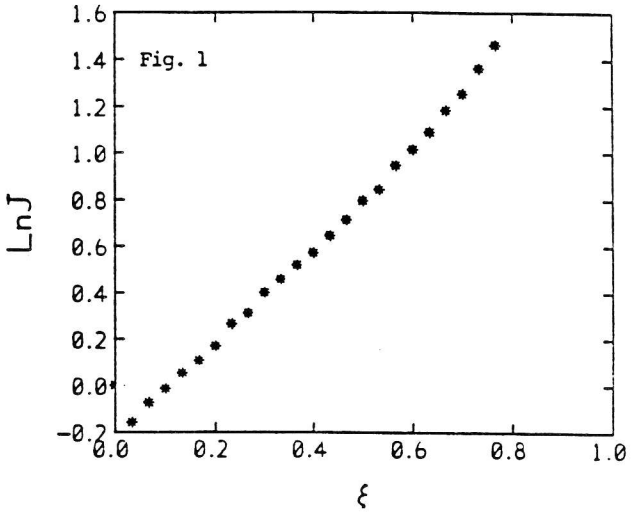
Titin from sheep skeletal muscle was isolated and partially purified in native form as described earlier (2). Only one titin band was observed on 3% SDS-PAGE although lower molecular weight contaminants, possibly including nebulin (ranging from 10-30% in various preparations based on stain uptake) were difficult to remove by published procedures.

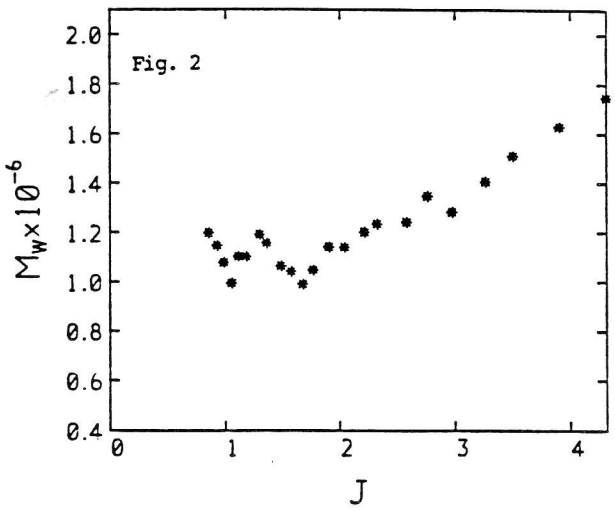
For the sedimentation equilibrium studies a Beckman Model E analytical ultracentrifuge was used, equipped with Rayleigh Interference Optics. The 'low speed' method was employed (3) and the (fringe) concentration at the meniscus, Ja was obtained by mathematical manipulation of the fringe data (3). The solvent used (pH=7.5) comprised 50mm Tris/HCl + 0.5M KCl + 1mM EDTA, and the initial cell loading concentrations of titin used were between ≈0.5 and 1.5 mg/ml. The partial specific volume used was that used by Trinick et al (2). Two types of molecular weight were sought (i) the weight average for the whole solute distribution, M_w^c and (ii) the point weight average, M_w as a function of (absolute fringe) concentration, J. M_w^c is obtained from extrapolation to the cell base of a particularly useful 'operational' point average, the star average, M^* and M_w values obtained by sliding strip quadratic fits to the observed fringe data (3).

RESULTS & DISCUSSION

Fig. 1 illustrates the dependence of Ln J on the radial displacement parameter $\xi = (r^2 - a^2)/(b^2 - a^2)$, r being the radial distance from the centre of the rotor and a and b the corresponding positions of the cell meniscus and base respectively.

The upward curvature of this plot is indicative of the presence of heterogeneity. Indeed, despite the low speed (2806 rpm) the optical registration of the fringes near the cell base was lost due to their steepness; the meniscus region was nonetheless not depleted: these observations would appear therefore to be indicative of the presence of aggregates which may or may not be in chemical equilibrium. M^* extrapolated to $\xi = 1$ yields a value of $(1.4 \pm .1) \times 10^6$ for M_w^c. This is a weight average over the whole distribution and will be affected by the presence of aggregates, and also by the presence of thermodynamic non-ideality, which is likely to be significant for this extended molecule (2).





From Fig. 2 a value of $M_w(J \to 0)$ = 950,000 ± 100,000 is obtained; if the heterogeneity is caused by a genuine self-association then $M_w(J \to 0)$ will represent the titin 'monomer' and will not be affected by non-ideality. The value so obtained is considerably higher than for other large polypeptides. (This result may be slightly lower than the 'true' molecular weight due to the presence of impurities). The heterogeneity illustrated in both figures is likely to represent an underestimate, since thermodynamic non-ideality tends to mask such effects. Indeed, a similar experiment on a preparation from rabbit muscle yielded a very similar value for $M_w(J \to 0)$ - ≈ 900,000 - but even less evidence of heterogeneity, perhaps because of the greater non-ideality.

Again, if the heterogeneity is due to a genuine self-association then this may account for the observations of Trinick et al (ref. 2 & personal communication) - using platinum shadowing electron microscopy - of the high tendency of titin to aggregate: the high concentrations during air drying specimens on to mica would seem a plausible explanation.

A more detailed picture of titin heterogeneity will be obtained from additional experiments using different loading concentrations and solvents (including dissociating). The ability to use point average molecular weights that are independent of 1st order non-ideal effects (4) is clearly advantageous, but requires the use of very precise fringe data: to this end we are currently equipping our Model E with a pulsed laser light source and adapting a laser gel scanner to provide improved accuracy in the off-line processing of the interference fringes.

1. Wang et al (1979) Proc.Nat.Acad.Sci.(USA) 76, 3398
2. Trinick et al (1984) J.Mol.Biol. 180, 331
3. Creeth & Harding (1982) J.Biochem.Biophys.Meth. 7,25
4. Roark & Yphantis (1969) Ann.NY Acad.Sci.164, 245