ON THE MOLECULAR WEIGHT OF MYOSIN

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The major, and sometimes the only, peak visible in a schlieren diagram of sedimenting 5-h extracted "myosin B" arises from myosin[1], and not, as once assumed[2], from the principal light-scattering substance in a myosin-B solution. In reaching this conclusion we have measured the molecular weight of this major component (which studies in the ultracentrifuge with u.v.-absorption optics have shown to be about 65 % of the total protein of 5-h myosin B[1]), and compared it with the molecular weight of separately prepared myosin, using both the newly developed approach to sedimentation equilibrium (or "ARCHIBALD") method[3,4,5] and the conventional sedimentation velocity diffusion techniques. Thus we have incidentally collected new ultracentrifugal data on the molecular weight of myosin, and here present these data in their own right because the molecular weight of myosin seems to continue to be controversial[6].

METHODS

Myosin B was prepared using a 5-h extraction of rabbit muscle mince with Weber-Edsall solution, and myosin, or "myosin A", was prepared by extracting muscle mince in the presence of ATP**. All preparations showed an acceptably high ATPase activity[2]; the myosin solution showed no turbidity drop upon addition of ATP, and the sedimentation of the principal component of myosin B was not affected by ATP. The solvent employed was 0.6 *M* KCl, adjusted to pH 7.0 with dilute HCl or KOH.

The ultracentrifuge was a Spinco Model E, equipped with a phase-plate schlieren diaphragm, and a Spinco RTIC system for rotor-temperature measurement and control. The solutions were refrigerated (about 5°) until just before use, and in runs of more than 90 min duration the rotor temperature was maintained at 5°. Short runs were carried out with the rotor at room temperature, after establishing that sedimentation patterns and coefficients are unchanged by such exposures. All runs were made in cells with plastic (Kel-F) centerpieces[2]. Plates were read with a Gaertner two-dimensional comparator. In using the Archibald method, measurements were made at the meniscus only; no artificial cell-base[5] was employed because preliminary examination showed evidence of surface denaturation of myosin at the interface between the solution and the silicone oil usually used as a cell base. For obtaining S *vs.* c data at very low concentrations ($c < 10^{-2}$ g/100 ml) the schlieren diaphragm was shifted from the center of the optical track, thus making possible the visualization of boundaries by using angles as low as 5° for the schlieren diaphragm. Also, in such measurements long (30 mm) optical path cells were employed. Diffusion coefficients were estimated from boundary spreading at about 5° by both area-height and inflection-point methods. The boundary was formed either in a synthetic boundary cell, or else it was formed in a regular cell, moved rapidly to the middle of the cell and then allowed to spread at low rotor speeds. The partial specific volume was taken to be 0.728[7].

* The opinions expressed in this article are those of the authors, and do not necessarily reflect the opinions of the Navy Department or the Naval Service at large (Statement added in accordance with U. S. Navy regulations).

** This preparation was devised and kindly supplied to us by Dr. JEAN BOTTS.

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RESULTS

The results of an Archibald-type experiment are illustrated in Fig. 1, where M_m, the weight-average molecular weight of the sedimenting system calculated at the meniscus, is plotted against time of centrifugation at 4200 r.p.m.*. Extrapolation of the (upper) myosin B curve to time zero shows that the initial weight-average molecular weight for the system is several million grams, as deduced from light scattering[2]. As centrifugation continues, however, the heavier components pull away from the meniscus, and the molecular weight falls, finally approaching asymptotically the molecular weight of the lightest species present, *viz.*, $(4.2 \pm 0.2) \cdot 10^5$ g. The myosin ("myosin A"), on the other hand (lower line), shows from the beginning a constant molecular weight, *viz.*, $(4.26 \pm 0.09) \cdot 10^5$ g. Clearly the lightest species present in myosin B is myosin, and we have here two closely agreeing estimates for the molecular weight of myosin.

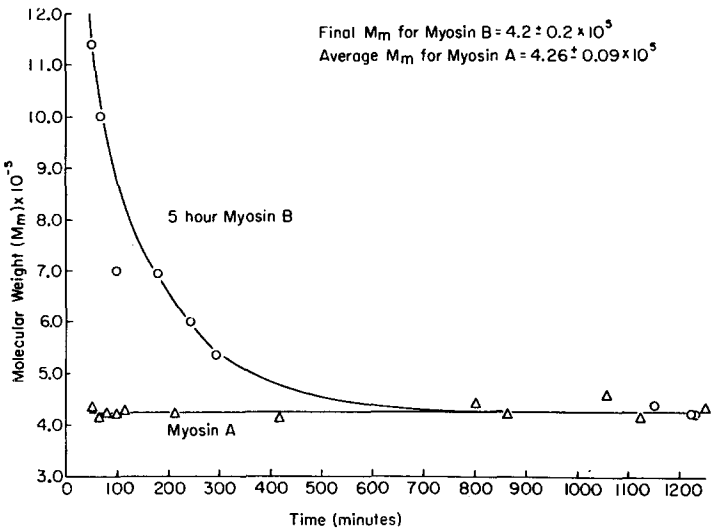

Fig. 1. Molecular weight of myosin (lower curve) and 5-h extracted myosin B (upper curve), measured at the meniscus by the "Archibald" approach to sedimentation equilibrium technique, as a function of time of sedimentation at 4197 r.p.m. and *ca.* 5°. Initial protein concentrations, 0.5 g/100 ml.

In Fig. 2 the S°_{20} of the major component of 5-h myosin B is plotted against the protein concentration**. S°_{20}, from extrapolation to infinite dilution, is 6.25 ± 0.15 S. It is interesting that when measurements at sufficiently low concentrations are made, the S_{20} *vs.* c curve does not swerve upward, as it does for many asymmetric substances (see also PORTZEHL *et al.*[8]).

D°_{20}, (D_{20} extrapolated to infinite dilution), could only be estimated crudely

* This rotor speed was chosen because the opposing sedimentation and diffusion fluxes of particles of molecular weight *ca.* $5 \cdot 10^5$ g are about equal in this gravitational field. Thus near the meniscus we can follow the approach to sedimentation equilibrium of particles of this size, while much larger particles are rapidly sedimented out of this region of the cell.

** These observations were made after a half hour of centrifugation at 52,640 r.p.m. Separate u.v.-absorbance measurements[1] show that under these conditions some 35% of the total protein, in the form of much heavier molecular-weight components, has sedimented completely to the base of the cell, thereby eliminating possible complications due to the Johnston-Ogston effect. Accordingly the concentrations shown are 65% of the initial total protein concentrations.

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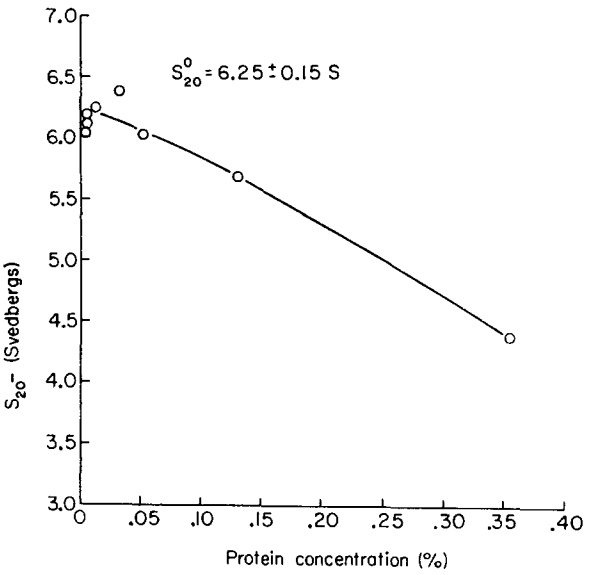

Fig. 2. Sedimentation coefficient (S_{20}) of the major ultracentrifugal component of 5-h myosin B, as a function of protein concentration.

from our experiments. Values of D_{20} obtained employing the synthetic boundary cell (at *ca.* 5000 r.p.m.) are probably too low despite our efforts (observation only after considerable centrifugation and only on the solvent side of the boundary) to eliminate heavy components. Observations made by centrifuging the boundary to the middle of a standard cell before permitting diffusion are free of the heavy-component complication, but involve relatively large zero-time corrections. Averaging results from both methods, D_{20} at $c \sim \cdot 35$ g/100 ml is approximately $1 \cdot 10^{-7}$ cm^2/sec. Assuming, by analogy with the behavior of S_{20} as a function of c, that D^0_{20} is about two-fifths higher, and combining this value with the above S^0_{20}, we obtain a conventional molecular weight estimate of $4 \cdot 10^5$ g, in reasonable agreement with that given by the Archibald method.

We feel that the Archibald method, on which our myosin molecular weight estimate of $4.2 \cdot 10^5$ g is primarily based, is well suited to the problem since, as applied here it involves measurement under conditions wherein the sedimentation flux is of the same magnitude as the diffusion flux, *i.e.*, very small. Thus the hydrodynamic complications which play a role in the concentration dependence of both S and D for asymmetric proteins are avoided here, and the dependence of molecular weight on concentration is given by the virial expansion[9]. At high (0.6 M KCl) ionic strength it can be expected, and indeed it is found[2], that the second virial coefficient for the myosins is essentially zero; therefore the molecular weight measured by the present technique should be practically identical with the molecular weight at infinite dilution.

Our numerical result, like those of several others (500,000 g by sedimentation-diffusion[10]; 650,000 g[11] and 530,000 g[12] by light-scattering), is low relative to the sedimentation[8]–diffusion[13] (838,000 g) and osmotic[13] (840,000 g) results of PORTZEHL *et al.*; however, our result is in line with such minimum molecular weight estimates as can be made from data on myosin sub-units.

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SUMMARY

By the Archibald approach to sedimentation equilibrium method, we have found a molecular weight of $4.2 \cdot 10^5$ g for either, (1) myosin (A) or, (2) the principal, (*ca.* 65% by weight), schlieren-visible component of 5-h extracted myosin B. This same value has also been estimated from the conventional sedimentation velocity-diffusion method, using sedimentation coefficients obtained at very low protein concentrations, and diffusion coefficients measured by observing boundary spreading in synthetic boundary cells. Although lower than hitherto reported molecular weights for myosin (A), this value is in line with minimum molecular weight estimates based on the present knowledge of myosin sub-units.

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Note added in proof

A condensed account of the foregoing results appeared on p. 14T, *Abstracts, 132nd Meeting, Am. Chem. Soc.*, New York, September, 1957. It is encouraging to note that since this paper has been in press, there have appeared preliminary reports of values for the molecular weight of myosin which agree closely with those reported here, *viz.*, the report of W. F. H. M. MOMMAERTS AND B. B. ALDRICH (*Science*, 126 (1957) 1294), who used the "Archibald method", and that of J. GERGELY (p. 46, *Abstracts, Biophys. Soc. Meeting*, Boston, February, 1958) who used light-scattering.

A STUDY OF THE α-KETO-ACIDS IN BLOOD

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INTRODUCTION

Several keto-acids are present in biological fluids and it is obvious that their individual quantitative estimation is necessary in order to understand their role in normal and pathological metabolic states.

A number of methods have been devised for their quantitative determination. The most recent among them is an enzymic procedure which is rapid and specific, but is limited, at the moment, to a few α-keto-acids[1,2].

The colorimetric determination of α-keto-acids, which is simple and fairly accurate, has been used for some fifteen years. The best known method, has been described by FRIEDEMANN AND HAUGEN[3] and consists in the colorimetric assay of the 2,4-dinitrophenylhydrazones of α-keto-acids in alcaline medium. These and other authors

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