*SUBUNIT STRUCTURE OF MYOSIN, II. HEAVY AND LIGHT ALKALI COMPONENTS**

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Previous work from this laboratory has indicated that in 5 *M* guanidine, rabbit skeletal myosin dissociates into a heavy component of molecular weight greater than 200,000 and a light component (~17%) of molecular weight about 46,000.[1] Myosin is also dissociated in alkaline solution, having at pH 10.7 a number-average molecular weight of 170,000 as determined from osmotic pressure.[2] Work by Kominz *et al.*[3] indicated that at pH about 10 myosin has, on sedimentation velocity, a main peak similar to that of the intact protein and a slow peak reported to represent 14–18 per cent of the total protein. The light component was fractionated using ammonium sulfate; its molecular weight was estimated as 29,000 from sedimentation-diffusion, and it was found to have a C-terminal end-group, isoleucine, identical with that of myosin.[4] The amino acid composition of this light component is essentially identical with that of a light component isolated from myosin in urea[3, 5] and, although the point was not evident at the time, is also similar to the amino acid compositions of H-meromyosin[6–8] and subfragment-1.[8, 9]

These findings have suggested the working hypothesis that myosin is composed of a fibrous core, which is dissociated in 5 *M* guanidine, and a number of light subunits of molecular weight 29,000–46,000 which are located at the head end of the molecule and are dissociated both in alkaline solution and 5 *M* guanidine.[10] The experimental findings on myosin in alkaline solution are critical to the hypothesis, and it is the purpose of the present study to characterize more fully the light and heavy components of myosin at high pH.

Materials and Methods.—Rabbit skeletal myosin was prepared by the method of Szent-Györgyi,[11] with modifications described elsewhere.[1, 10] Reduction and carboxymethylation were carried out at 4°C in 0.5 *M* KCl–0.025 *M* Tris at pH 8.0.[1] The meromyosins were prepared by the method of Lowey and Holtzer.[12] For the studies in alkaline solution, the samples were dialyzed for 12–24 hr against the appropriate buffer (0.4 *M* KCl, 0.1 *M* Na_2CO_3 at pH 11.0; or 0.4 *M* KCl, 0.1 *M* K_2HPO_4 titrated with KOH to pH 12.5).

Ultracentrifugal studies were carried out at 4°C throughout in a Beckman model E analytical ultracentrifuge.[1] High-speed sedimentation equilibrium experiments were done by the method of Yphantis[13] and analyzed using interference data to displacements (J) as low as 0.1–0.2 fringes. Corrections for Wiener skewing were negligible.

Protein concentrations were determined from optical densities measured with a Zeiss PMQ-II spectrophotometer and corrected for Rayleigh scattering. A value for $E^{1\%}_{280}$ of 5.60 was used.[14] The absorption at 280 mμ was constant within ± 2% from pH 6.5 to pH 12.5, despite a shift in the absorption maximum from 279 mμ at pH 6.5 to 290 mμ at pH 12.5.

The refractive index increment of myosin, determined from synthetic boundary experiments using capillary double-sector centerpieces, was 0.184 ml/gm at pH 6.5, 11.0, and 12.5. This value was assumed to be identical for all dissociated components. The partial specific volume was assumed to be 0.720 ml/gm at 4°C.[15]

Cellulose acetate electrophoresis was performed in a Beckman microzone electrophoresis system at room temperature.

Results.—Myosin at pH 6 to 8: Sedimentation velocity experiments performed on

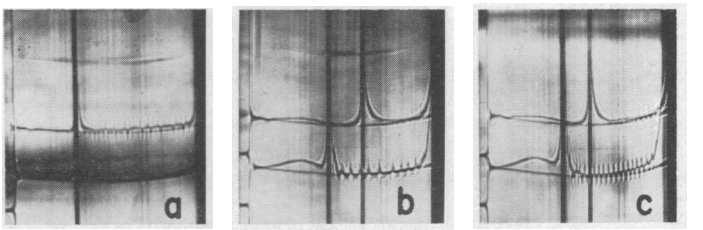

FIG. 1.—Schlieren patterns of myosin (XIII) at 56,100 rpm, phase plate angle 70°. (*a*) 232 min. *Upper*, pH 9.5, 10.7 mg/ml; *lower*, pH 6.5, 13.7 mg/ml. (*b*) 297 min, pH 11.0. *Upper*, CM, 9.0 mg/ml; *lower*, CM, 17.5 mg/ml. (*c*) 301 min, pH 12.5. *Upper*, CM, 9.0 mg/ml; *lower*, 18.4 mg/ml.

myosin in 0.5 *M* KCl at pH 6.5–8.0 invariably showed a single sharp peak. The sedimentation coefficient, $S^{\circ}_{20,w}$ was 6.3*S*. A small leading edge, representing slight aggregation, was occasionally noted (Fig. 1*a*). Sedimentation equilibrium experiments, to be described fully elsewhere, indicated negligible contamination with low (5,000–50,000) molecular weight material. A molecular weight for myosin in the range 490,000–520,000 was found, in agreement with other studies[16–18, 10]

Myosin at alkaline pH: The effect of increased pH on the sedimentation behavior of myosin is shown in Figure 1. At pH above 9.5, there is a widely spread boundary which trails the main peak. Enhanced aggregation at alkaline pH is evidenced by a leading edge to the main peak at $C_0 > 5$ mg/ml or a small leading peak at lower concentrations. In experiments at $C_0 > 9$ mg/ml, there is obvious convection in the plateau region, which may be related in part to aggregation.

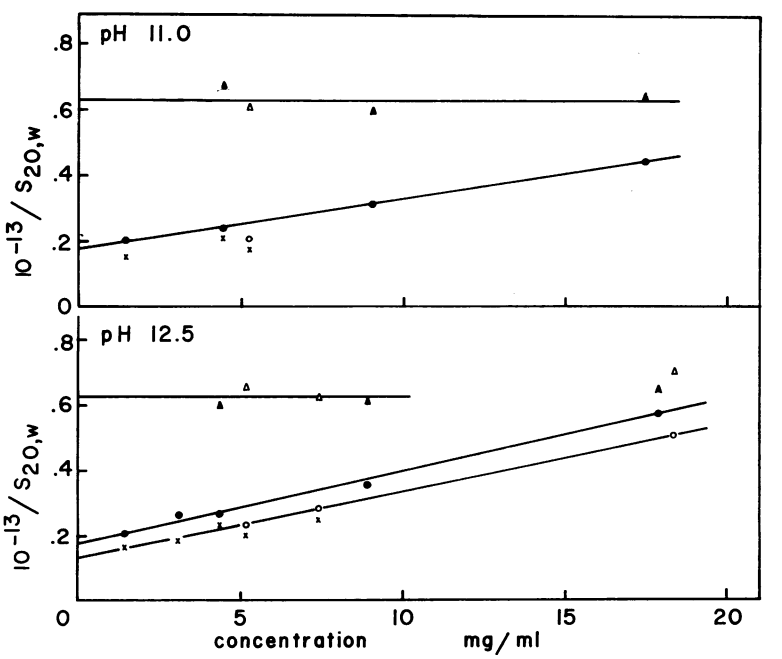

FIG. 2.—Reciprocal of $S_{20,w}$ against total protein conc. for alkali components of myosin (XIII). *Upper*, pH 11.0; *lower*, pH 12.5. △, Myosin LAC; ○, HAC; ▲, CM myosin LAC; ●, HAC; ×, HAC aggregate. Lines denote average for LAC and least squares for HAC.

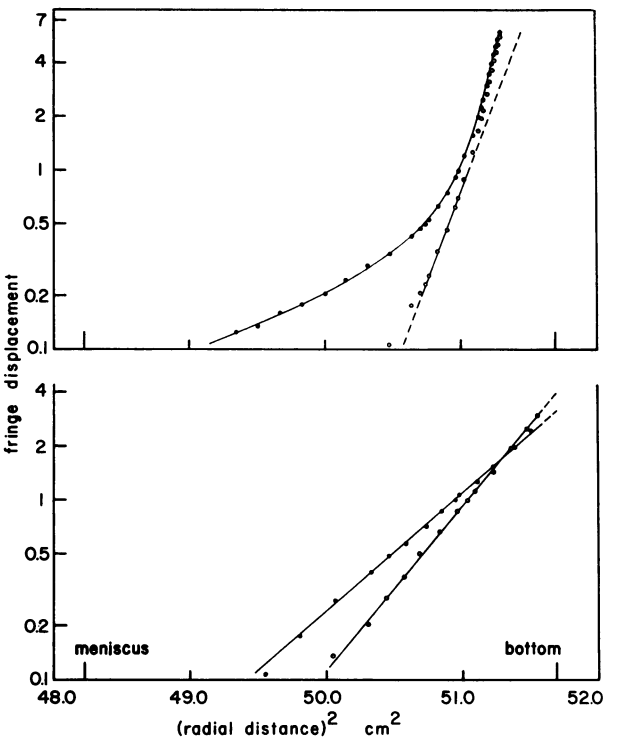

FIG. 3.—Fringe displacement on a logarithmic scale against r^2 for a sedimentation equilibrium expt. on reduced and carboxymethylated myosin (XI) at pH 11.0. *Upper:* 13,340 rpm for 60 hr; ●, observed fringe displacement from meniscus position; O, HAC (calculation described in text). *Lower:* ●, observed data at 35,690 rpm for 24 hr; and O, at 42,010 rpm for 30 hr. For the lower graph, the dotted lines show extrapolations to r_b^2. The following symbols are used in the text: r, radial distance; r_m, radial distance to meniscus; r_b, radial distance to bottom; J_m, fringe displacement at meniscus; J_b, fringe displacement at bottom.

The main peak, representing the heavy alkali component (HAC), shows a marked concentration dependence, greater at pH 12.5 than at pH 11.0 (Fig. 2). The HAC from carboxymethylated (CM) myosin yields values for $S^\circ_{20,w}$ of 5.70S at pH 11.0, and 5.63S at pH 12.5. In the absence of prior carboxymethylation, the $S^\circ_{20,w}$ values of the main peak are higher and the extent of aggregation is somewhat greater.

The trailing peak, representing the light alkali component (LAC), shows no significant concentration dependence at $C_0 < 10$ mg/ml and yields average values for $S^\circ_{20,w}$ of 1.6S at pH 11.0 and pH 12.5 (Fig. 2). There is no significant difference related to prior carboxymethylation.

The relative proportion of the alkali components was determined from the schlieren areas in four experiments at 4–9 mg/ml (Table 1). At lower concentrations, the schlieren displacements for the LAC are too small for reliable measurements; and at higher concentrations, convection and incomplete resolution of the components preclude meaningful results. The LAC comprises 15–21 per cent of the total protein after correction for radial dilution, a result consistent with the range of 14–18 per cent reported earlier.[3] Corrections for both radial dilution and the Johnston-Ogston effect[19] were carried out by the method of Trautman *et al.*,[20] and the results indicate that the LAC actually comprises only 12.2 per cent (±0.4% SE) of the total protein.

Sedimentation equilibrium experiments were performed on myosin at pH 11.0–12.5. In the experiment shown in Figure 3, the preparation exhibits obvious heterogeneity at equilibrium at 13,340 rpm. At equilibrium using field strengths seven- and tenfold greater (35,600 rpm and 42,010 rpm), the heavy component is effectively

TABLE 1

FRACTION PER CENT OF ALKALI COMPONENTS OF MYOSIN FROM SEDIMENTATION VELOCITY*

Conc. (mg/ml)	pH	Time (min)	$A_L\left(\frac{r_L}{r_m}\right)^2$	$A_H\left(\frac{r_H}{r_m}\right)^2$	LAC (%), radial dilution correction	$\gamma - 1$	A_L°	A_H°	LAC (%), radial dilution and Johnston-Ogston correction
4.5	11.0	96	0.113	0.585	16.2	0.150	0.098	0.600	14.0
		112	0.110	0.585	15.8	0.166	0.095	0.600	13.7
		192	0.104	0.556	15.8	0.177	0.088	0.572	13.3
4.4	12.5	96	0.123	0.599	16.8	0.302	0.095	0.627	13.2
		112	0.132	0.586	18.1	0.309	0.101	0.612	13.8
		144	0.106	0.609	14.8	0.160	0.091	0.624	12.7
8.9	12.5	189	0.295	—	20.6†	0.580	0.187	1.246†	13.0†
		221	0.308	—	21.5†	0.605	0.192	1.241†	13.4†
		285	0.275	—	19.2†	0.635	0.168	1.265†	11.7†
		317	0.279	—	19.4†	0.643	0.170	1.263†	11.9†
9.1	11.0	185	0.228	—	15.6†	0.661	0.137	1.322†	9.4†
		233	0.213	—	14.6†	0.444	0.148	1.311†	10.1†
		281	0.231	—	15.8†	0.494	0.154	1.305†	10.6†
		313	0.226	—	15.5†	0.497	0.151	1.308†	10.3†
		466	—	1.305‡	—	—	—	—	—
Average (± standard error)					17.1 (±0.6)				12.2 (±0.4)

Symbols: A_L, observed area of LAC; A_H, observed area of HAC; r_L, radial distance to LAC peak; r_H, radial distance to HAC peak; A_L°, corrected area of LAC; A_H°, corrected area of HAC; γ, correction factor (see ref. 21)

* Carboxymethylated myosin (XIII).

† Based on total protein conc.

‡ Area determined after boundary-spreading at 4000 rpm for 135 min.

removed. The graph of log J against r^2 for the residual light component is linear and yields a molecular weight of 19,270 at both speeds. Since the light component is distributed ideally throughout most of the cell, its average concentration, J_0, is readily calculated:[21] $J_0 = [2(J_b - J_m)]/[\sigma(r_b^2 - r_m^2)]$. The value of σ[13] is calculated from the linear slope; the value of J_b is estimated from a short extrapolation to r_b^2; and that of J_m is effectively zero. From the total concentration as determined in a synthetic boundary experiment, the relative proportion of light component may then be estimated, and in this experiment is 10.0 per cent at 35,690 rpm and 10.8 per cent at 42,010 rpm.

Other equilibrium experiments indicate comparable results at pH 11.0 and pH 12.5 for myosin and CM myosin (Table 2). For the four experiments, the average molecular weight for the light component is 20,200 (±450 SE), and its average proportion is 11.6 per cent (±0.5% SE) of the total protein, in close agreement with the value of 12.2 per cent from sedimentation velocity. Taking the molecular weight of myosin to be in the range 490,000–520,000, the results indicate that a molecule of myosin contains on the average 3.0 (±0.3) subunits of molecular weight about 20,200.

In the experiment shown in Figure 3, the properties of the heavy component were determined at the lowest speed (13,340 rpm), by subtracting from the observed data the contribution of the light component as calculated from the data at the higher speeds. For the residual components the graph of log J against r^2 is linear from 0.2 to 0.8 fringes, and yields a molecular weight for the HAC of 418,000. The increasing slope of the graph above 0.8 fringes is indicative of aggregation.

Fractionation of the alkali components: A fractionation procedure based on solubility differences was developed as follows: at 4°C the alkaline solution of myosin was titrated to pH 7–8 with 1 M KH_2PO_4, rapidly diluted with 15–20 vol of distilled water, centrifuged at 10,000 g for 15 min, and separated into supernatant and pre-

TABLE 2

MOLECULAR WEIGHT AND FRACTION PER CENT OF ALKALI COMPONENTS OF MYOSIN FROM SEDIMENTATION EQUILIBRIUM

Preparation	pH	Rotor speed (rpm)	Light Alkali Component: Mol wt	Light Alkali Component: Per cent	Heavy alkali component ($M \times 10^{-5}$)*
Myosin (XI), CM†	11.0	13,340	—	—	4.2 (±0.4)
		35,690	19,270	10.0	—
		42,010	19,270	10.8	—
Myosin (XIII), CM	12.5	39,440	19,800	12.8	—
Myosin (XIII)	12.5	39,400	21,400	11.9	—
Myosin (XIII), CM	11.0	39,400	20,200	11.4	—
Average (± standard error)			20,200 (±450)	11.6 (±0.5)	
First-cycle supernatant (XI), CM†	11.0	41,950	19,540	100.	—
		52,550	19,050	100.	—
First-cycle precipitate (XI), CM†	11.0	11,230	—	—	4.3 (±0.6)‡
Second-cycle precipitate (XII), CM†	11.0	11,240	—	—	4.6 (±0.3)§
		39,400	22,000	2.8	—
Third-cycle precipitate (XIV), CM†	11.0	12,750	—	—	11.1 (±1.2)§
		10,560	—	—	9.5 (±0.4)§
		39,400	20,080	1.2	—
First-cycle precipitate (XI), CM†, in 5 *M* guanidine:HCl	5.7	20,350	—	—	2.1 (±0.1)§
		37,010	22,100	6.0	2.1 (±0.5)§

* ± Estimated error in calculation.
† Preparation reduced prior to carboxymethylation.
‡ Corrected for LAC, assuming 6% of 20,200 molecular weight component.
§ Corrected as described for expt. of Fig. 3.

cipitate fractions. The supernatant, after dialysis against distilled water and lyophilization, was found to contain only LAC (Fig. 4). From sedimentation velocity experiments at pH 11.0, the precipitate fraction contained HAC and about 6.5 per cent LAC. After two additional cycles of the above procedure, the LAC content of the precipitate fraction was reduced to 0.6–1.2 per cent.

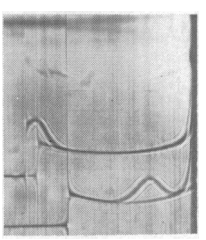

FIG. 4.—Schlieren pattern of LAC from pooled first- and second-cycle supernatant, pH 7.0, 50,740 rpm for 47 min, phase plate angle 70°. *Upper*, 3.4 mg/ml; *lower*, solvent layered over 3.4 mg/ml protein solution at 5,000 rpm using capillary double-sector centerpeice.

The LAC exhibited at pH 7.0 a single peak on sedimentation velocity (Fig. 4), with $S^{\circ}_{20,w} = 1.9S$, and contained negligible RNA contamination (orcinol test). In sedimentation equilibrium experiments on LAC from first-cycle supernatant, the plot of log J against r^2 was linear and yielded a molecular weight of 19,300.

Heterogeneity of the LAC was demonstrated by electrophoresis on cellulose acetate. As illustrated in Figure 5, LAC in first-cycle supernatant from CM myosin showed four electrophoretic components: a fast band and a slow band that may be split (*a*, *b*, and *i*) were invariably present, and a wide band of intermediate mobility was occasionally present (*c* and *g*). Prior reduction did not cause significant differences (*a* and *b*). In general, each preparation showed only three of the components, and preliminary experiments on LAC in second- and third-cycle supernatant fractions have not indicated additional components. The electrophoretic patterns for LAC from myosin without carboxymethylation were less well resolved, and appeared

to show two (*e*) or perhaps three (*f*) bands. It is uncertain in this preliminary work whether the differences are due to differential fractionation of similar subunits in all preparations, or to genetic variations.

Sedimentation equilibrium experiments on the precipitate fractions indicated a molecular weight for the HAC of 433,000 in first-cycle precipitate and 458,000 in second-cycle precipitate (Table 2). There was increasing aggregation of the HAC with each successive fractionation, and an experiment on third-cycle precipitate indicated a molecular weight for the HAC of order 1×10^6 (Table 2).

Heavy alkali component in 5 M guanidine: First-cycle precipitate from CM myosin was dialyzed against 5 *M* guanidine·HCl at pH 5.7 for 1 week at 4°C. Sedimentation velocity experiments show a main peak similar to that of myosin in 5 *M* guanidine[1] and a marked diminution in the trailing component. On sedimentation equilibrium, the sample contained a component (6%) of molecular weight 22,100, a result similar to the findings on first-cycle precipitate prior to dialysis against 5 *M* guanidine (Table 2). After correction for the low-molecular-weight component, the heavy component had a molecular weight of 2.1×10^5, a value approximately one-half that of the HAC prior to dialysis against 5 *M* guanidine.

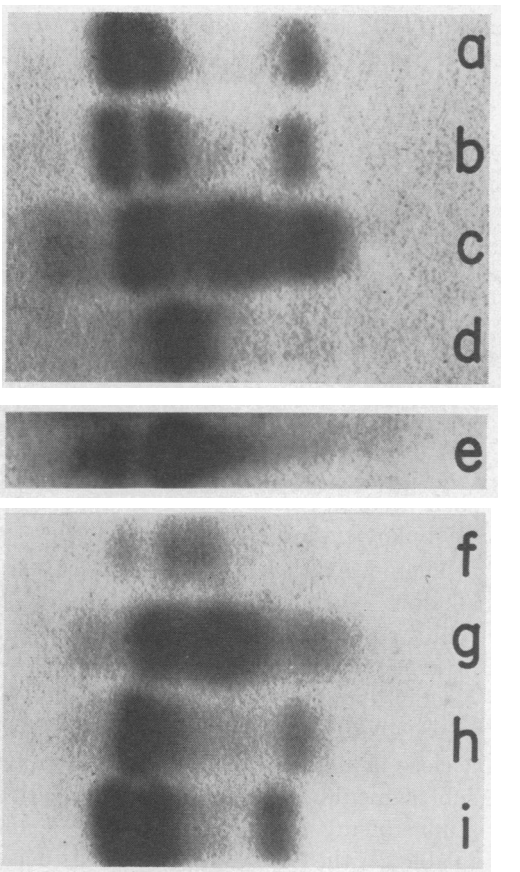

FIG. 5.—Cellulose acetate electrophoresis on first-cycle supernatant (LAC). (*a*)–(*d*) and (*f*)–(*i*) run for 1 hr at 225 v in pH 8.6, 0.075-ionic-strength barbital; (*e*) run for 45 min at 500 v in pH 8.6, 0.025-ionic-strength barbital (stained with ponceau S). Origin beyond left edge, anode to right. (*a*), From CM myosin (XII), pH 11.0; (*b*), from reduced and CM myosin (XII), pH 11.0; (*c*), from reduced and CM myosin (XI), pH 11.0; (*d*) and (*e*), from myosin (XI), pH 11.0; (*f*), from myosin (XIII), pH 11.0; (*g*), from CM myosin (XIII), pH 12.0; (*h*), from CM myosin (XIII), pH 10.5; (*i*), same as (*a*).

Meromyosins at alkaline pH: As shown on sedimentation velocity (Fig. 6), H-meromyosin contained a trailing component at pH 11.0 which was not present at pH 8.0 and which represented somewhat less than 19 per cent of the protein. On sedimentation equilibrium, H-meromyosin at pH 11.0 contained a component (10%) of molecular weight 6,550, presumably similar to the protomyosins;[22] a component (19%) of molecular weight 22,400; and a residual component of molecular weight greater than 2.0×10^5. In contrast, L-meromyosin at pH 8.0 and pH 11.0 showed a single peak on sedimentation velocity, and on sedimentation equilibrium at pH 11.0, contained a protomyosin-like component (10%) of molecular weight 7,400; a main component of

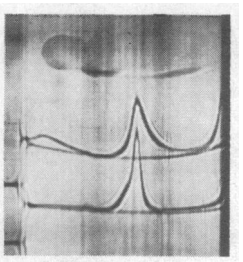

FIG. 6.—Schlieren patterns of H-meromyosin (from CM myosin) at 56,100 rpm for 136 min, phase plate angle 70°. *Upper*, pH 11.0; *lower*, pH 8.0; both 7.4 mg/ml.

molecular weight about 100,000; and no evident component with intermediate molecular weight. These findings indicate that the LAC is localized in H-meromyosin and that a molecule of H-meromyosin of molecular weight 340,000[12, 7, 17] contains (on the average) three subunits of molecular weight about 22,000.

Discussion.—The data indicate that a molecule of rabbit skeletal myosin contains (on the average) three light subunits which dissociate at alkaline pH from its H-meromyosin end. The light subunits are monodisperse on sedimentation, have an average molecular weight of 20,200, a frictional ratio f/f_0 of 1.41, and are soluble at low ionic strength (0.05) at pH 7–12.5. There is a C-terminal residue, isoleucine[3] (confirmed in this laboratory), and electrophoretic heterogeneity with approximately three major bands. In view of their hydrodynamic and solubility properties, these subunits are termed the *g* (globular) subunits.

There are several lines of evidence, in addition to their actual demonstration in alkaline solution, which suggest that *g* subunits are present in all preparations of rabbit skeletal myosin: (1) The C-terminal residue of myosin[4, 3, 23] and H-meromyosin[23] has been identified as isoleucine. This amino acid is an end group of the *g* subunits[3] and, from work to be fully described elsewhere, not an end group of the heavy alkali component. (2) In preparations from different laboratories, there is a close similarity between the amino acid compositions of the *g* subunits[3] and of subfragment-1,[8, 9] a small enzymatically active part of H-meromyosin. (3) From studies on myosin in 5 *M* guanidine[1, 10] and on first-cycle precipitate in 5 *M* guanidine (Table 2), the light components dissociated at alkaline pH and in 5 *M* guanidine would appear to be identical.[24] It is then likely that light components earlier reported to be dissociated from myosin by urea[2, 5, 25] are composed, at least in part, of *g* subunits.

The *g* subunits are dissociated at a pH somewhere between 9.5 and 10.5; over the same range there is an abrupt change in Ca^{++}-activated myosin ATPase, from a maximum (pH 9.5) to its complete loss (pH 10.5).[26] Subunit interactions involving the *g* subunits may thus be essential to the enzymatic activity of myosin.

The HAC is presumably the fibrous core of myosin, having a molecular weight of about 430,000, a frictional ratio of 3.63, and the general solubility properties of intact myosin. Preliminary data on first-cycle precipitate in 5 *M* guanidine and studies on myosin in 5 *M* guanidine[1, 10] indicate dissociation of the HAC into two subunits of average molecular weight 215,000. These are termed the *f* (fibrous) subunits.

From these findings and electron microscopic[27] and X-ray diffraction[7] studies on myosin, it would seem that a reasonable model for myosin is that of an axial core of two *f* subunits that form a double helix and extend into a globular head region containing three *g* subunits.[28]

Of great interest and under present study is the exact relationship of the subunit interactions to the actin-binding property and the enzymatic activity of myosin.

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[28] The model clearly includes major elements of previous models, namely, the multichain axial skeleton and small subunits of Tsao,[2] and the 2-chain α-helix with globular head of Lowey and Cohen;[8] and it is also consistent with experimental findings earlier interpreted as a 3-chain system by Kielley and Harrington.[14] The 3-chain model is based on a molecular weight for myosin in guanidine of 187,000–206,000, from methods that are essentially weight-average.[10] According to the model here proposed, the myosin molecule on complete dissociation would have a weight-average molecular weight of 191,000 (196,000 with association among the three g subunits).