light chains. The latter explanation is supported by the observation that the alkali-1 light chain exchanges into myosin more easily that alkali-2 light chain[10]. As the alkali-2 light chain is more easily removed from S-1 than alkali-1 light chain, in the rest of the experiments described here, the S-1 and heavy chains were prepared from S-1(A2).

The actin-activated ATPases of both S-1 and S-1 heavy chain have hyperbolic dependences on actin concentration (Fig. 2). In preparation A of Table 1, after correcting for residual S-1(A2), the V_{max} of S-1 heavy chain was 49% that of native S-1(A2) and 67% that of NH_4Cl-treated S-1(A2). In these assay conditions, the V_{max} of S-1(A1) is about half that of S-1(A2), but when the ionic strength is increased to 0.04 M, the V_{max} of S-1(A1) increases to that of S-1(A2)[13]. Preparation B of Table 1 shows the effect of ionic strength on the actin-activated ATPase of the S-1 heavy chain. Although the recovery of actin-activated ATPase was lower than in preparation A, the actin-activated ATPase activities were still much higher than could be accounted for by residual S-1(A2). The V_{max} of the S-1 heavy chain did not increase significantly when the ionic strength was increased. At both ionic strengths, the K_{app} of the S-1 heavy chain was between those of S-1(A2) and S-1(A1).

Further purification of the heavy chains was achieved by removing residual S-1(A2) on the immunoadsorbent column in the absence of NH_4Cl. No alkali-2 light chain was detectable on SDS-polyacrylamide gels (see Fig. 1). The EDTA-, Ca^{2+}- and actin-activated ATPases of pure S-1 heavy chain were all 30% of those of native S-1(A2). The decreases in activities, most noticeable in the EDTA-ATPase, probably reflect the instability of the heavy chain during the isolation procedures.

To check that the S-1 heavy chains did not contain fragments of the alkali light chains (produced by proteolysis during the isolation procedures) which were not visible on Laemmli-type SDS gels[17], the lysines of an S-1 heavy-chain sample were modified in urea with ^{3}H-formaldehyde and $NaCNBH_3$ (ref. 14). The sample was run on an SDS-urea gel capable of resolving small peptides[15]. There were no counts in the area of small peptides which could be attributed to digestion products of the alkali light chains nor were any bands detectable by Coomassie blue staining.

The binding of the S-1 heavy chain to F-actin was examined by ultracentrifugation (see Table 2). Centrifugation in the absence of actin showed a slight aggregation of the S-1 heavy chains. (Other experiments also showed that the S-1 heavy chains tend to aggregate more readily than does S-1.) After correcting for aggregation, approximately the same fractions of S-1 heavy chain and S-1(A2) bound to F-actin in the absence of ATP, but a lower fraction of S-1 heavy chain was released by ATP.

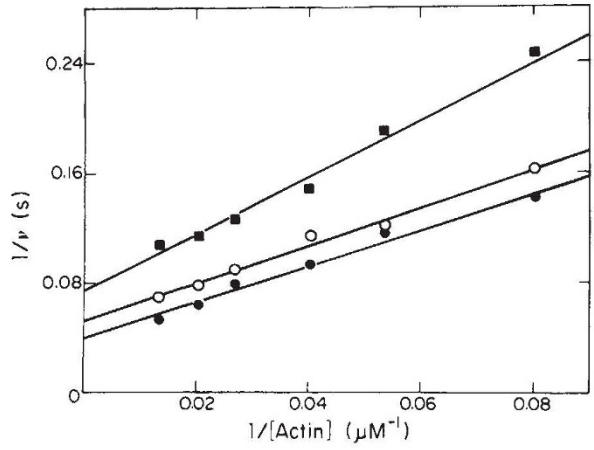

Fig. 2 Actin-activated ATPases. Assays were performed as described in Table 1 legend; v is the observed rate of ATP hydrolysis by S-1 in the presence of actin, minus that of S-1 alone. ●, S-1(A2); ○, NH_4Cl-treated S-1(A2); and ■, S-1 heavy chain containing 10% S-1(A2).

Table 2 Actin binding of S-1(A2) and S-1 heavy chain preparations

	Fraction bound	
	−ATP	+ATP
S-1(A2)	0.91 (0.02)	0.09 (0.03)
NH_4Cl-treated S-1(A2)	0.82 (0.08)	0.05 (0.03)
S-1 heavy chain (10–15% S-1(A2))	0.82 (0.06)	0.17 (0.09)

S-1 heavy chain or S-1(A2) (1 μM) was mixed with 36 μM F-actin in 0.1 M KCl, 2 mM $MgCl_2$, 10^{-4} M DTT and 5 mM imidazole, *p*H 7.0 at 4° C. Some samples also contained 1.0 mM ATP. After centrifuging the samples at 150,000*g* for 30 min, the supernatants were carefully removed and aliquots electrophoresed on SDS-polyacrylamide gels. The gels were stained with Coomassie brilliant blue and the intensity of the heavy-chain bands determined using a scanning gel densitometer. When centrifuged in the absence of actin, 80% of the S-1 heavy chain and 90% of the S-1(A2) and the NH_4Cl-treated S-1(A2) remained in the supernatant. These values were used as references for determining the fraction of S-1 bound to F-actin. Values are the mean (±s.d.) of three different S-1 heavy-chain preparations.

These results show that the alkali light chains are not essential for either actin binding or ATP hydrolysis, and that the ATP and actin binding sites are located on the myosin heavy chains. However, the alkali light chains may stabilize the myosin conformation, as the heavy-chain preparations seem to be unstable. It has been reported that the isolated heavy chain of the non-filamentous *Acanthamoeba* myosin 1B is fully active[16]. As this myosin has many unique properties, it was not possible to extrapolate this observation to other myosins. However, as fast skeletal muscle myosin is a 'typical' myosin, it is likely that the ATP and actin binding sites of other myosins will also reside on the heavy chains.

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Erratum

In the letter 'Lamellar-zonal bone with zones and annuli in the pelvis of a sauropod dinosaur' by R. E. H. Reid, *Nature* **292,** 49–51 (1981), the sentence beginning on the first line of page 51 should read 'Some material in the literature cited[7] could also be interpreted differently, for example if bone figured by Seitz (Figs 53, 54 of ref. 14) from the theropod *Allosaurus* is thought to show lamellar-zonal structure'.