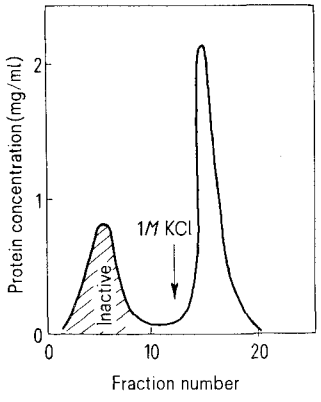
Practically all the protein exhibiting ATPase activity was adsorbed and eluted as a sharp peak on increasing the salt concentration. SDS gel electrophoresis of the eluted protein gave 3 bands corresponding to 110,000, 90,000 and 70,000 dalton polypeptides similar to those observed with skeletal HMM S-1[8] and 2 bands corresponding to the 17,000 and 20,000 dalton light chains, reported to be associated with smooth muscle myosin[9]. When examined by sedimentation velocity analysis, the preparation behaved as a homogeneous protein.

The specific ATPase activities of the eluted protein at pH 7.5 and 37 °C were 2.5 μmole/mg/min for Ca^{2+}-activation (in 0.6 *M* KCl, 5 m*M* $CaCl_2$), 1.5 μmole/mg/min for K^+-ATPase (in 0.6 *M* KCl, 10 m*M* EDTA). These activities correspond to a roughly 2-fold increase as compared to the parent myosin. In presence of $MgCl_2$ (5 m*M*) at low ionic strength (~0.1) the ATPase activity was very low (2.5 nmole P_i/mg/min) as measured with the enzyme linked assay described in[10]. It could be activated up to 5-fold after addition of rabbit skeletal actin exceeding the concentration of S-1 up to 100-fold. Moreover, subsequent work had shown that the activity of acto-S-1 proved to be dependent on the presence of trace Ca^{2+} and could be greatly reduced by addition of EGTA[11]. The activation by actin was similar in degree to that observed previously in actin-activated vascular smooth muscle ATPase[12].



Affinity chromatography of arterial HMM S-1.
Purification by agarose ATP of arterial myosin sufragment-1 obtained by papain digestion of actomyosin. 10 ml of crude arterial actomyosin (10 mg/ml) in 0.5 *M* KCl, 20 m*M* imidazole pH 7, and 0.1 m*M* DTT was digested by papain at 1/200 weight ratio for 10 min at 25 °C and separated by centrifugation at 100,000 *g* for about 3 h after the addition of 10 m*M* Ag-ATP. It was dialyzed thoroughly (to remove Mg-ATP) against high ionic strength solution and then against the equilibrating buffer containing 30 m*M* KCl, 10 m*M* imidazole pH 7, 0.1 m*M* DTT and 1 m*M* EDTA. Any precipitated protein was removed by centrifugation at 30,000 *g* for 1 h. The crude subfragment was applied on a 0.9×12 cm Seph · adipic hydrazide-ATP column, previously equilibrated by the above solution, and the protein was eluted stepwise by 1 *M* KCl in the same buffer. The fraction volume was 2 ml.

On rechromatographing the dialyzed protein, complete adsorption occurred and elution by a KCl gradient gave a sharp peak centered at 0.2 *M* KCl if EDTA was included in the buffers. A somewhat wider peak around 0.6 *M* was eluted when 3 m*M* $MgCl_2$ was present. Similar effect of $MgCl_2$ on the affinity to agarose hydrazide ATP of skeletal muscle myosin was ascribed previously to the metal ion-linked splitting by myosin of the bound ATP[13].

The method described is simple and avoids the difficulties involved in preparation of pure arterial myosin as well as undefined, unspecific overdigestion of the type observed after trypsin treatment[4]. The examined ATPase properties of the HMM S-1 obtained were undamaged, inspite of the fact that some excess of papain had to be applied. The effect of limited proteolysis on the solubility of crude arterial myosin suggests that it can be a tool to explore the involvement of yet unknown factors in this property.

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CONGRESSUS

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Corrigendum

L. A. MITSCHER, J. V. JUVARKAR and J. L. BEAL: *Solacasine, a New Steroidal Alkaloid from Solanum pseudocapsicum Possessing Antimicrobial Activity*, Experientia *32*, 415 (1976). Formulae 1, 2 and 3 erroneously depict a 15, 16 fusion of the heterocyclic rings to the steroid skeleton. The correct fusion is, of course, **16, 17**.