stained with Giemsa. This can be clearly demonstrated in the European field vole *Microtus agrestis*, where most of the late replicating constitutive heterochromatin is confined to the large sex-chromosomes. If BrdU is present at the beginning (end) of the S-phase, these heterochromatic regions are darkly (faintly) stained (Figure 1).

Similarly, the facultative heterochromatic *X*-chromosome in mammals is distinguishable from its euchromatic homologue which can be especially useful for analysis of the inactivation patterns of abnormal *X*-chromosomes (Figure 2). Generally, a close correlation exists between the banding patterns of the chromosomes after BrdU incorporation at early (late) S-phase and G-banding (R-banding), which has been already described[5,9,10]. Thus, this BrdU technique has some clear advantages over the ^{3}H-TdR autoradiography: it is cheaper, quicker and provides a better resolution for examining the timing of chromosomal DNA replication. As in most cell lines the duration of G_2 phase is rather uniform, the time of BrdU removal or application is fairly constant, irrespectively of any differences in generation times.

Furthermore the technique can be used instead of G-banding, resulting in a higher yield of well banded metaphases, as well as in the additional identification of both *X*-chromosomes in the female.

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Affinity Chromatographic Preparation of Arterial Heavy Meromyosin Subfragment-1

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Summary. Heavy meromyosin subfragment-1 (HMM S-1) was prepared by papain digestion of arterial myosin or actomyosin and was purified by agarose-ATP affinity chromatography. Proteolysis of crude arterial myosin suspensions was preceded by solubilization. HMM-S-1 thus obtained consisted mainly of a 90,000 dalton polypeptide and fully retained the K^+- and Ca^{2+}-ATPase of the parent myosin. Its affinity to agarose-ATP was comparable to that of skeletal muscle HMM S-1.

The study of arterial and other smooth muscles is faced with unsolved preparative problems originating from a peculiar solubility behavior of its actomyosin and from difficulties in separation of actin from its myosin preparation. Since agarose-ATP affinity chromatography technique proved useful for the purification of active fragments of myosin from striated muscles[3] we were tempted to try and apply this procedure in order to prepare pure and well defined heavy meromyosin (HMM S-1) from arterial muscle. A preparation of this subfragment has already been reported by Huriaux[4] who overdigested myosin with trypsin instead of papain, which proved to be so specific and useful for the preparation skeletal myosin s_1 (c.f.[5]).

Arterial actomyosin was prepared from pig carotides according to Russel[6]. Crude arterial myosin was obtained by centrifugation of a solution of actomyosin (10 mg/ml) in 0.6 *M* KCl, 0.1 m*M* dithiothrietol (DTT), 50 m*M* *Tris* pH 7.6 and 10 m*M* Mg-ATP for 6–12 h at 100,000 *g*. Sepharose adipic hydrazide-ATP (agarose-ATP) was prepared as previously described[7].

Two approaches to the preparation of arterial HMM S-1 were followed:

1. Papain digestion (10 min, 25 °C) of myosin (5 mg/ml) in suspension at low ionic strength (30 m*M* KCl, 10 m*M* imidazole pH 7 and 0.1 m*M* DTT) and purification of the active fragment by adsorption to Sepharose adipic-hydrazide-ATP after removal of insoluble protein by centrifugation (100,000 *g*, 45 min); 2. Papain digestion of arterial actomyosin solution, separation of actin by centrifugation for 45 min at 100,000 *g*, removal of salt, ATP and insoluble protein by dialysis plus centrifugation and application to the affinity column (for details see legend to the Figure).

In the course of examination of the effect on the yield and activities of HMM S-1 of increasing papain: myosin ratios, it was observed that papain at a low level (papain: myosin 1/8000–1/2000 w/w) solubilized most of the ATPase activity within 10 min at 25 °C. SDS gel electrophoresis of the 100,000 *g* supernatant after this period revealed that most of the myosin was in the form of the intact 200,000 dalton heavy chains of myosin, i.e. that solubilization was not due to proteolysis. This unusual behavior prompted us to apply papain in excess (1/200) to insure complete digestion of the myosin. The same papain: myosin ratio was found adequate also in the digestion of actomyosin.

The Figure describes the affinity chromatography step in the purification of HMM S-1 derived from actomyosin.

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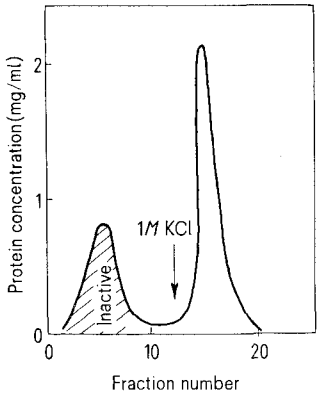
Practically all the protein exhibiting ATPase activity was adsorbed and eluted as a sharp peak on increasing the salt concentration. SDS gel electrophoresis of the eluted protein gave 3 bands corresponding to 110,000, 90,000 and 70,000 dalton polypeptides similar to those observed with skeletal HMM S-1[8] 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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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**.