

Myosin isozymes in vertebrate smooth muscles

G. Beckers-Bleukx, G. Maréchal

Université Catholique de Louvain, Département de Physiologie, B-1200, Bruxelles, Belgium

Electrophoreses of smooth muscle extracts (chicken gizzard; guinea-pig taenia coli; canine and human stomach and colon) in non-dissociating pyrophosphate gels yield 1 to 4 zones according to the composition of the extracting solution or the temperature of the electrophoretic runs. Organs extracted in a Guba-Straub solution with 10 mM $\text{Na}_4\text{P}_2\text{O}_7$, an acid solution (pH 6.5), yield 1 zone only if electrophoresed at 4° C, but two if electrophoresed at 0° C (except human colon, which yielded only 1). If 10 mM ATP is added to the Guba—Straub solution, 3 zones are observed. The two slowest zones have the same mobility than those observed if the extraction is made in an ATP-free solution. We called the 3 zones: G2, G1 and G0, by order of increasing mobilities. G1 is sometimes replaced by two closely spaced zones (G12, G11). 10 mM ATP has no effect on organs extracted in a Weber-Edsall solution with 10 mM $\text{Na}_4\text{P}_2\text{O}_7$, a basic solution (pH 8.8): two zones are observed, corresponding to G2 and G1. Reelectrophoresis on SDS-12% PAA gels show two light chains (17 and 20 kDa) for G2, G1 and G0, on SDS-6% PAA: heavy chains of 230 kDa for G2, 200 kDa for G0 and a mixture of the two for G1. Antifilamin added to the extract at an approximate molar ratio of 4/1 depresses G2 by 80 to 100% and G1 by 50%. Anti-smooth muscle LMM binds to Western blots of G2, G1 and G0. These results indicate that contrary to our earlier proposal, [Beckers-Bleukx & Maréchal, *Eur. J. Biochem.* 152, 207-11 (1985)] G2 and G1 probably do not contain myosin isoforms but myosin-filamin complexes of different molar ratio (1/4 and 1/1 respectively) whilst G0 contains myosin but no filamin. Since filamin-free myosin is only observed in the presence of ATP in the extracting solution, it may be that filamin binds to unphosphorylated myosin, and not to phosphorylated myosin.