The influence of cations on the polymerization of actin and actin in the presence of α-actinin/filamin.

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Filamin and α-actinin are both, actin binding and cross-linking proteins found in various muscle and non-muscle cells, which in their dimeric form, play an important role in cell architecture. Here, we have examined the influence of ionic strength and monovalent/divalent ions on the lag phase, rate and amplitude of polymerizing actin in the presence/absence of filamin/α-actinin.

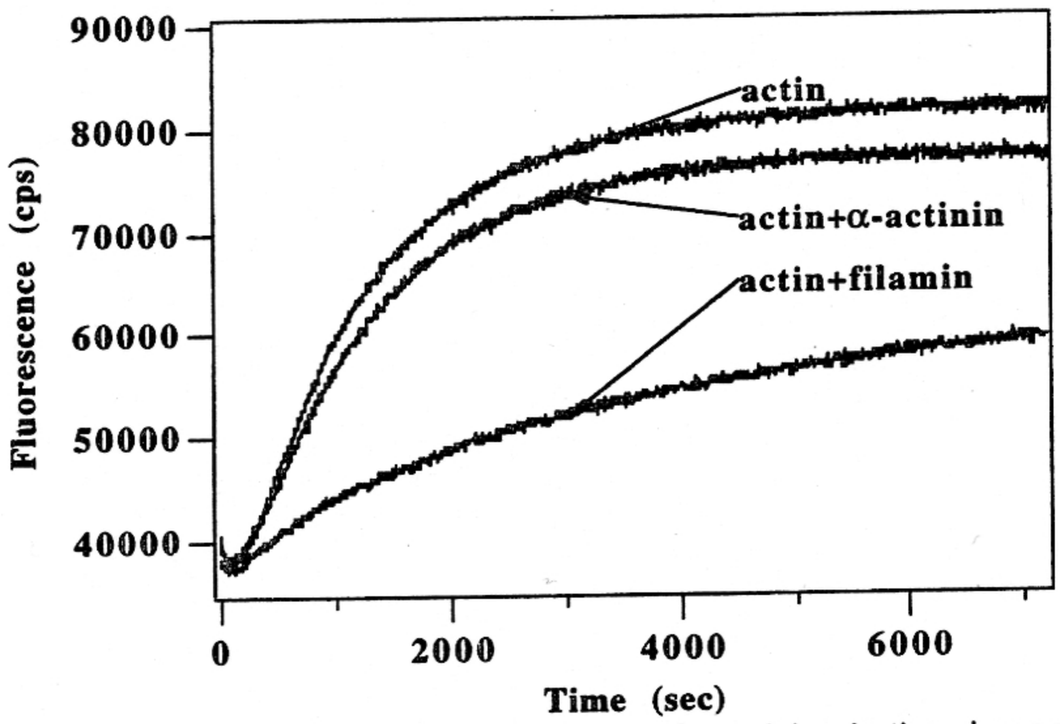

Fig.1 Fluorescent traces of 3 μM actin polymerizing in the absence of filamin/α-actinin and 2.73 μM actin in the presence of 0.27 μM filamin/α-actinin. Buffer: *50 mM KCl*, 2 mM $MgCl_2$, 0.2 mM $CaCl_2$, 1 mM EGTA, 2 mM Tris-HCl, pH 7.5, 0.2 mM ATP, 0.2 mM DTT, 0.005% NaN_3 and 20°C.

Filamin was isolated by the method of Shizuta et al. [1], and α-actinin was prepared as described by Craig et al. [2] from smooth muscle with modifications [3]. Actin was purified from acetone powder obtained from rabbit muscle [4] and labelled with 7-chloro-4-nitrobenzeno-2-oxa-1,3-diazole (=NBD) [5]. All kinetic experiments were performed in a SPEX Fluorolog 1680 double spectrophotometer, exciting at 480 nm and emitting at 530 nm.

The use of NBD fluorescence of labelled G-actin allows the determination of polymerizing actin. Fig. 1 shows typical polymerization traces of 15 % NBD-actin and 85 % unlabelled actin mixed in the presence/absence of filamin/ α-actinin (actin : filamin/α-actinin at a molar ratio of 10 : 1).

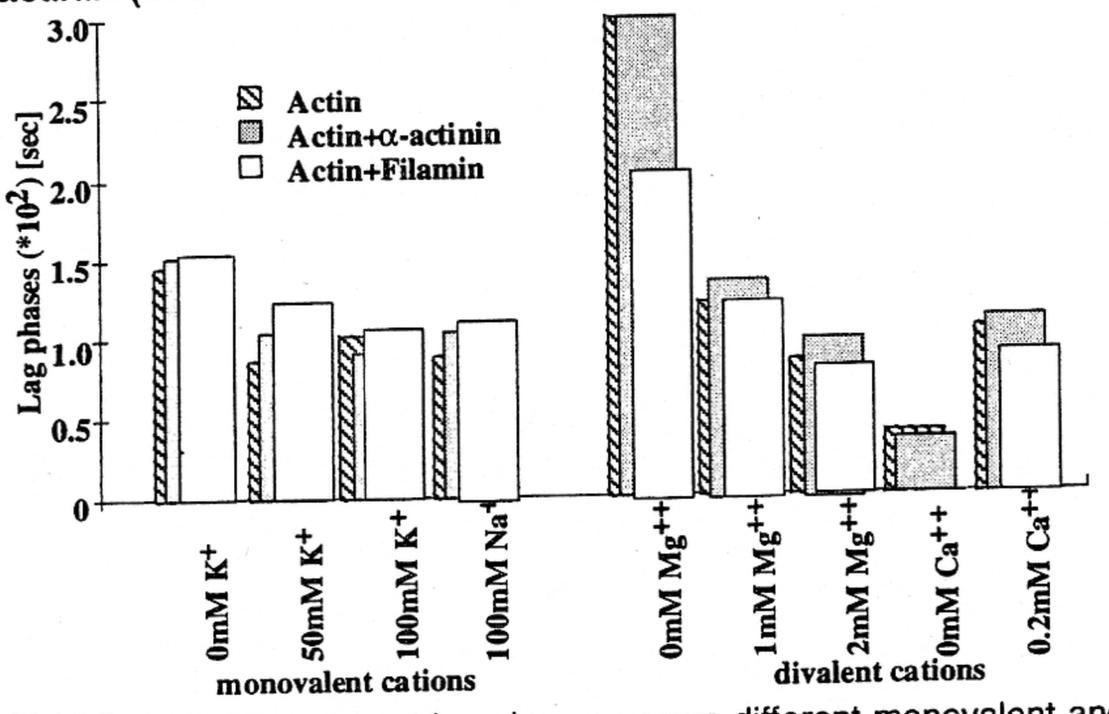

Fig. 2 Plot of fluorescent lag phases versus different monovalent and divalent cations. Standard deviation is ±25 % (n=3) for all measurements. Experimental conditions: 3 μM actin or 2.73 μM actin and 0.27 μM filamin/α-actinin. *Normal buffer:* 100 mM KCl, 2 mM $MgCl_2$, 0.2 mM $CaCl_2$, 2 mM Tris-HCl, pH 7.5, 0.2 mM ATP, 0.2 mM DTT, 0.005% NaN_3; where indicated, these ions were used with other ions of the *normal buffer* kept constant.

The lag phases for actin in the presence/absence of filamin/α-actinin in Fig. 2 under the various ionic conditions, determined by a polynominal fit, are comparable when considering the high standard deviation (SD ±25%). Thus, an impressive decrease in lag is observed for all species with an increase in KCl and $MgCl_2$ concentrations.

The polymerization rates for actin in the presence of α-actinin at various monovalent/divalent ion concentrations show a slight decrease, however, in the presence of filamin a significant decrease compared to pure actin (Fig. 3).

The fluorescence amplitudes for actin in the presence of α-actinin in Fig. 4 show a similar trend under these buffer conditions as observed for the lag, with the exception that divalent ions have no special influence, yet the presence of filamin shows a marked reduction under all conditions.

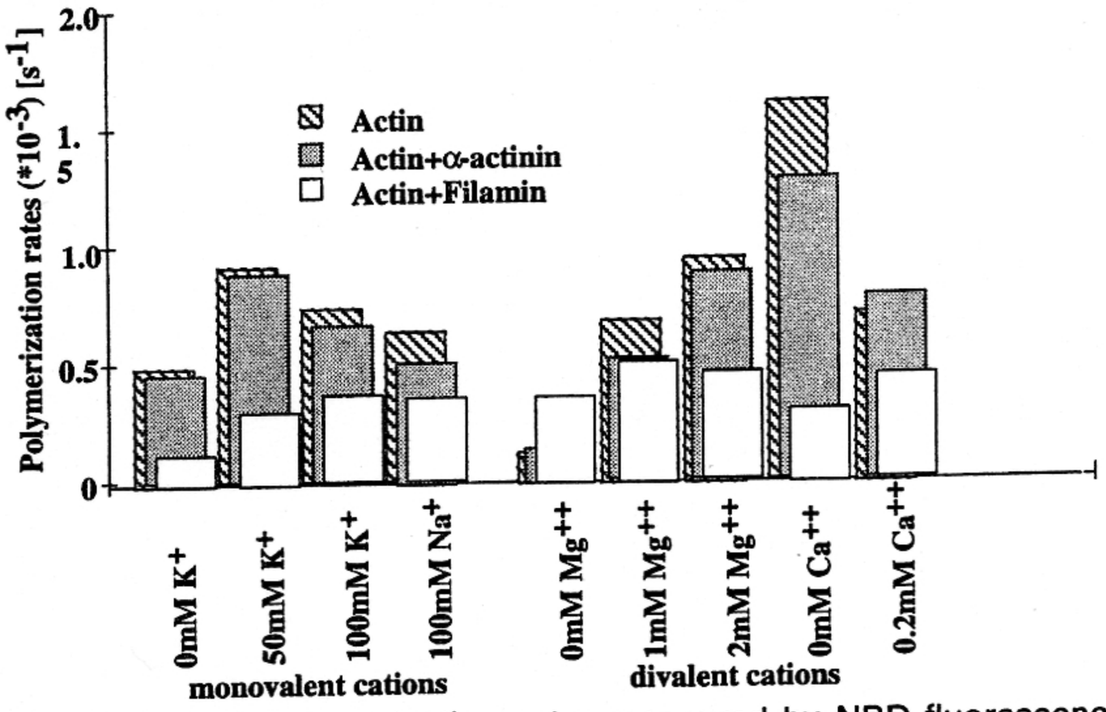

Fig. 3 Plot of polymerization rates measured by NBD fluorescence versus different monovalent and divalent cations. Standard deviation is ±20 % (n=3) for all measurements. Experimental conditions: 3 μM actin or 2.73 μM actin and 0.27 μM filamin/a-actinin. Buffer as in Fig. 2.

Results show a significant reduction in polymerization rate and fluorescence amplitude for actin/filamin compared to actin and actin/α-actinin. Since the NBD label detects changes in fluorescence intensity linearly with the concentration of polymerized actin, we determined the following free (G)-actin concentrations after polymerization by DNase-I binding assay: pure actin = 15.5%; actin/α-actinin = 20.6%; actin/filamin = 9.5%. These findings agree with amplitude changes for actin and actin/α-actinin, but not for actin/filamin. Further elaborate studies are needed to determine the subtle differences of the actin cross-linkers.

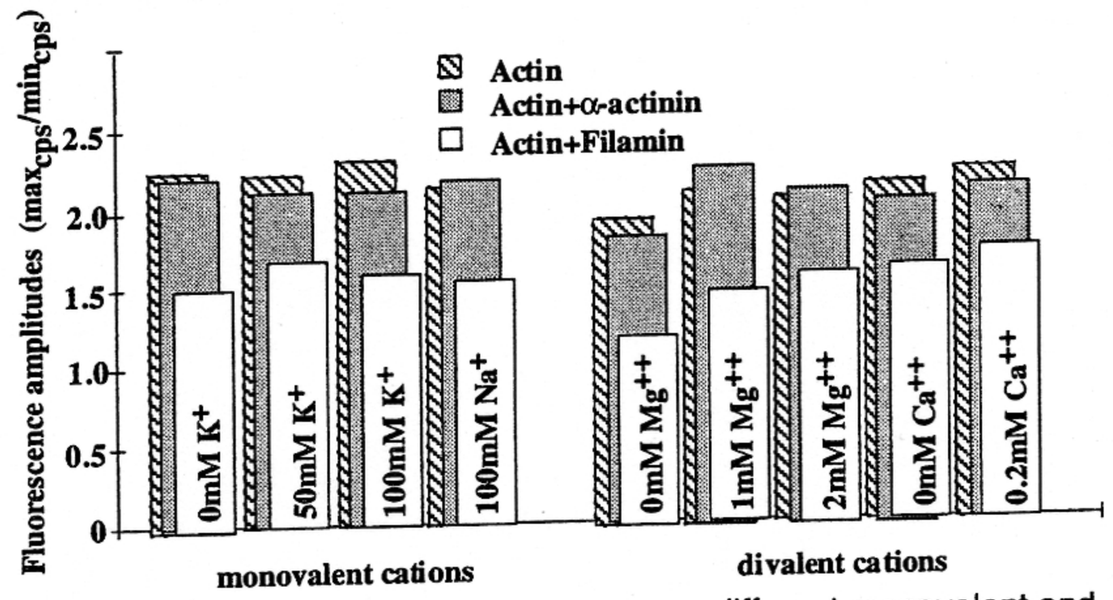

Fig. 4 Plot of fluorescent amplitudes versus different monovalent and divalent cations. Standard deviation is ±4 % (n=3) for all measurements. Experimental conditions: 3 μM actin or 2.73 μM actin and 0.27 μM filamin/ α-actinin. Buffer as in Fig. 2.

This work was supported by DFG Is 25/7-1, SFB 266/C5 and NATO CRG 950666 grants.

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