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The exchange of actin-bound calcium with various bivalent cations

Several authors have recently reported that purified actin contains 1.0–1.5 moles of bound calcium per 60000 g of actin[1–5]. BARANY and coworkers[1,6] found that Ca^{2+} is the only bivalent cation bound by actin. According to these authors Mg^{2+} can exchange partially with the bound calcium, whereas Zn^{2+} can be only additionally bound to actin without any exchange with bound calcium. On the other hand, some authors presented more or less direct evidence that actin-bound calcium can be at least partially replaced by such bivalent cations as Mg^{2+} (refs. 5, 7–9), Zn^{2+} (ref. 7) or Mn^{2+} (refs. 7, 9).

In the present study the exchanges of various bivalent cations with calcium bound to G-actin has been examined. In agreement with BARANY *et al.*[4,6] the present experiments show that bound calcium completely exchanges with free Ca^{2+} at 0.1 mM concentration. According to our results this reaction seems to proceed faster than the polymerization of actin since in 1 mM Ca^{2+}, even in the presence of 0.1 M KCl, *i.e.* under conditions when polymerization takes place, bound calcium also completely exchanges with free Ca^{2+}.

In Table I the exchange of ^{45}Ca bound to G-actin with various bivalent cations

TABLE I

THE EXCHANGE OF ACTIN-BOUND CALCIUM WITH VARIOUS BIVALENT CATIONS

Crude actin, extracted at 0° (ref. 10), polymerized with 0.1 M KCl and purified according to MOMMAERTS[11], was incubated with 0.1 mM $^{45}CaCl_2$ (ref. 4) in 0.2 mM ATP and 2 mM Tris–HCl (pH 8.0) for 15 min and then treated for 3 min at room temperature with Dowex-50W (0.08–0.1 mequiv/ml of actin). After removal of the resin actin (2–3 mg/ml) was incubated for 1 h with 0.1 mM or 1.0 mM salts of bivalent cations and then treated again with Dowex-50W. After removal of the resin, radioactivity was measured using a Chicago Nuclear Corp. gas-flow counter with "Micromil" window*, protein was determined by the biuret method and viscosity was measured in Ostwald viscometer at 20°, several hours after addition of an equal volume of 0.2 M KCl. Only plastic vessels were used and all pipettes were washed with EDTA and subsequently with deionised water. For the experiments performed in order to check the release of bound ATP during the exchange, G-actin, after preincubation with [^{14}C]ATP (Schwarz BioResearch*), was incubated for 1 h with salts of bivalent cations. After subsequent treatment with Dowex-50W and Dowex-1 X8 and removal of the resins radioactivity and protein concentration were measured in the solutions.

Salt added	*Concentration of the added salt*							
	0.1 mM				*1 mM*			
	*% of exchange***		*% of exchange***		*η red in 0.1 M KCl*		*Bound nucleotide counts/min/mg actin*	
	Expt. 1	*Expt. 2*	*Expt. 3*	*Expt. 4*	*Expt. 3*	*Expt. 4*	*Expt. 5*	*Expt. 6*
$CaCl_2$	100	100	100	100	8.5	6.7	1152	1086
$MnCl_2$	83	100	95	97	7.9	8.3	1108	1017
$CdCl_2$	71	75	102	104	9.8	7.8	1073	1171
$MgCl_2$	42	40	65	80	6.3	9.2	1189	920
$ZnCl_2$	23	25	68	80	7.2	7.4	—	835
$CoCl_2$	—	22	—	80	—	9.7	1216	925
$NiSO_4$	24	17	75	68	7.8	9.4	1138	1028
$SrCl_2$	19	15	7	25	7.0	9.2	1082	918
$BaCl_2$	0	0	0	0	6.2	8.8	1123	1065
None	—	—	—	—	8.3	7.5	1117	1110

*Obtained as a gift from the Rockefeller Foundation.
** $CaCl_2$ = 100.

is presented. It can be seen that Mn^{2+} and Cd^{2+}, at least at 1 mM concentration, exchange completely with bound calcium. On the other hand, Mg^{2+}, Zn^{2+}, Ni^{2+} and Co^{2+} exchange only partially and the extent of this exchange depends on the concentration of the added cation. Somewhat unexpectedly, it has been found that Sr^{2+} exchanges with bound calcium only slightly and Ba^{2+} does not exchange at all.

We have not yet determined directly the amount of any of the investigated cations bound to actin after their incubation with this protein. However, in spite of removal of both calcium released during incubation and the excess of added cations, the actin preparations obtained are fully active *i.e.* they still contain bound nucleotide and they polymerize in 0.1 M KCl. This fact strongly supports the view that in our experiments a replacement of bound calcium by other cations takes place.

The results presented above support the recent observations[5,7-9] that Mg^{2+}, Mn^{2+}, and Zn^{2+} can replace bound calcium but, on the other hand, they are in disagreement with the results of BARANY *et al.*[4,6]. Furthermore, our experiments show that the number of bivalent cations which can replace bound calcium is much greater than it has been known so far. The exchange of bound calcium occurs only when bivalent cations (even at higher concentrations which cause a polymerization) are added to G-actin but, in agreement with BARANY *et al.*[6], it does not take place when actin had been previously polymerized[6]. This was probably the reason why these authors[4] did not observe any exchange of bound calcium with free Mg^{2+} when they homogenized F-actin pellets in 1 mM $MgSO_4$ and 0.1 M KCl.

On the basis of the present experiments one may assume that in investigations performed so far in which G-actin was polymerized with 1 mM $MgCl_2$ and 0.1 M KCl, or partially polymerized with 0.6–0.8 mM $MgCl_2$, one had actually to deal with a mixture of Ca-actin and Mg-actin. This fact might have some influence on various physico-chemical determinations performed on actin.

The results of our experiments point to the possibility to obtain actin preparations containing in a bound form other bivalent cations instead of calcium. The properties of such actin preparations and the amount of the cations found as bound to actin are now under investigation.

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