SHORT COMMUNICATIONS

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The divalent cation bound to actin and thin filament

It is known that actin binds specific divalent cations[1–4]. G-actin prepared by MOMMAERTS' method[5] ordinarily contains about 1.5 mole Ca^{2+} per 60000 g of actin[2,6], that is, 1.0 mole per 40000 g of actin. F-actin ordinarily binds less than 1.5 mole of Ca^{2+} because of the release of bound Ca^{2+} after polymerization[6]. Ca^{2+} bound to G-actin is easily exchanged for other Ca^{2+} or other divalent cations in the solvent[3,7–9]. On the other hand, Ca^{2+} bound to F-actin is not normally easily exchanged[3,7]; however, sonic vibration can render Ca^{2+} exchangeable[7]. These characteristics of divalent cations are very similar to those of nucleotides.

Divalent cations as well as nucleotides have been considered to be essential for maintaining polymer structure; when they are removed by EDTA treatment, dialysis, *etc.*, G-actin can be denatured and never polymerized. However, in view of the discovery that Ca^{2+} or nucleotides can be removed by prolonged dialysis without destroying the polymer structure of F-actin[10], and that G-actin, thus freed of them, can be polymerized in a sucrose solution[11], we consider that divalent cations and nucleotides are not necessary for the maintenance of the polymer structure but only for the maintenance of polymerizability of G-actin. Here, in addition to the examination of release of divalent cations from F-actin, we measure the amount of Ca^{2+} and Mg^{2+} bound to thin filament and myosin B.

We compared the release of Ca^{2+} and Mg^{2+} from F-actin (Fig. 1). Ca^{2+} in Ca-F-actin (F-actin which has bound Ca^{2+}) was removed faster than Mg^{2+} in Mg-F-actin

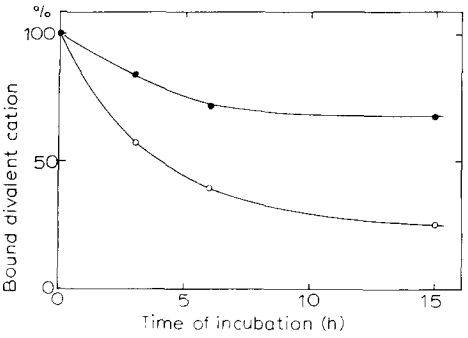

Fig. 1. Release of Ca^{2+} and Mg^{2+} from F-actin. F-actin was obtained by polymerization of G-actin prepared from rabbit skeletal muscle essentially according to the method of MOMMAERTS[5] with slight modifications[9]. Ca-F-actin and Mg-F-actin were obtained by sonication in $CaCl_2$ and $MgCl_2$, respectively, and by treatment on Dowex 50-X8 (200–400 mesh, K^+-type, 1/10 weight of solution) to remove excess divalent cations from the starting material[9]. F-actin was incubated in a temperature bath, and an aliquot of the solution was treated with Dowex 50 at the times shown to remove free Ca^{2+} and Mg^{2+} released from F-actin. The Ca^{2+} + Mg^{2+} content of action was about 1 mole per 40000 g. Actin, 1.4 mg/ml; KCl, 50 mM; Tris–HCl (pH 8.0), 10 mM; at 37°. ●—●, Mg-F-actin; ○—○, Ca-F-actin.

(F-actin which has bound Mg^{2+}). The half-life times of release were 3.9 h and 11 h at 37° for Ca^{2+} and Mg^{2+}, respectively. After 15 h, no decrease of flow birefringence of the F-actin solution was observed, indicating that the F-actin concentration was unchanged. According to the fact that the binding constant of Ca^{2+} is much larger than that of Mg^{2+} (refs. 8, 9), we might expect a faster release of Mg^{2+} than Ca^{2+}; however, the contrary is the case. This suggests that in the cyclic reaction of destruction and recombination of the bond, the release reaction is faster in the case of Ca-F-actin, while the binding reaction is stronger in the case of Mg-F-actin.

Natural F-actin which was prepared without the use of any depolymerization process or organic solvent treatment has more Mg^{2+} than Ca^{2+}, as will be shown later. Both were released in a way similar to the above-mentioned Straub-type F-actin, although the rates were somewhat low. From these findings, together with the fact that *in vivo* there is less free Ca^{2+} than Mg^{2+} (ref. 4), the divalent cation bound *in vivo* to F-actin is considered to be Mg^{2+}.

Next, we analysed the divalent cation content of natural F-actin, myosin and myosin B. The result is shown in Table I. Natural F-actin contains more Mg^{2+} than Ca^{2+} in a total amount of about 1 mole/40000 g actin. Natural F-actin is prepared mainly by the method of HAMA, MARUYAMA AND NODA[12]. In this method, high ionic strength HASSELBACH–SCHNEIDER solution[13] which contained Mg^{2+} was used to remove myosin from myofibril, possibly allowing the contamination of natural F-actin by Mg^{2+}. However, after this treatment, no additional divalent cations were employed. We could expect little exchange reaction with Mg^{2+} and or Ca^{2+} in F-actin during preparation because the removal of myosin was carried out at 0° for 3 h, and the half-life of exchange is more than 24 h under these conditions[4].

The divalent cation contents of myosin and myosin B were compared. The values are not extremely reliable, but they are consistent with other reports[4,14]. We assume that myosin B is composed of 210000 g of myosin and 60000 g of actin, *i.e.*, a weight ratio of 3.7:1 (ref. 15). The difference in the divalent cation contents is considered to be due to the actin in myosin B. This value suggests that actin contains Mg^{2+}, and half as much Ca^{2+}, the total amount being about 1.5 moles. This analysis

TABLE I

DIVALENT CATION CONTENT OF MUSCLE PROTEINS

Natural F-actin was prepared mainly by the method of HAMA, MARUYAMA AND NODA[12] (see text). Myosin B was prepared by SZENT-GYÖRGYI's method[19]. Myosin was prepared according to the method described by PERRY[20], with a slight modification. To remove free divalent cation, the sample solution was treated on Dowex 50-X8 (200–400 mesh, K^+-type)[9]. Divalent cation was measured by YANAGISAWA's method[18], with a slight modification, by using ethyleneglycol-bis-(β-aminoethyl ether)-*N,N'*-tetraacetic acid for the determination of Mg^{2+}.

Sample	*Ca^{2+} content*	*Mg^{2+} content*	*Total*
Natural F-actin	0.31 mole/ 60000 g	1.22 mole/ 60000 g	1.53 mole/ 60000 g
	0.68 mole/ 60000 g	1.01 mole/ 60000 g	1.69 mole/ 60000 g
Myosin B	1.5 ± 0.2 mole/270000 g	1.0 ± 0.2 mole/270000 g	2.5 ± 0.4 mole/270000 g
Myosin	0.9 ± 0.2 mole/210000 g	0.2 ± 0.2 mole/210000 g	1.1 ± 0.4 mole/210000 g
Actin in myosin B (myosin B — myosin)	0.6 ± 0.4 mole/60000 g	0.8 ± 0.4 mole/60000 g	1.4 ± 0.8 mole/60000 g

does not consider the divalent cation content of new protein in myosin B, such as actinin, tropomyosin and troponin. However, determination of the actin content of myosin B is difficult, and therefore, a definitive discussion is not possible.

There is no direct evidence for the content of divalent cation in thin filament, but the divalent cation in F-actin *in vivo* can be considered to be entirely Mg^{2+} (1 mole/40000 g of actin) in view of the release of Ca^{2+} from F-actin and the lack of free Ca^{2+} *in vivo*. Ca^{2+} in ordinary G-actin and even Ca^{2+} in natural F-actin or in actin of myosin B may come from other tissues such as granules or reticulum through an exchange reaction during preparation due to the strong affinity of Ca^{2+} for actin[6,8,9].

It is known that Mg^{2+} accelerates the polymerization rate and that Ca^{2+} slows it down[7,16] and also that ATP accelerates and ADP decelerates it[17]. Considering these facts, divalent cations as well as nucleotides may be rate regulators, but we have no evidence to show the role played by Mg^{2+} or Ca^{2+} in muscular contraction.

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