Biochimica et Biophysica Acta, 484 (1977) 169—176

BBA 68224

DUAL EFFECT OF Ca^{2+} ON ULTRASONIC ATPase ACTIVITY AND POLYMERIZATION OF MUSCLE ACTIN

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(Received March 1st, 1977)

Summary

Millimolar concentrations of Ca^{2+} stimulate actin polymerization whereas micromolar concentrations of Ca^{2+} depress polymerization. This latter effect leads to a reduction of ATPase (ATP phosphohydrolase, EC 3.6.1.3) activity of actin during sonication at low Mg^{2+} concentrations and in the absence of KCl. In the presence of KCl (90 mM) there is activation of ATPase activity by micromolar Ca^{2+} concentrations. These Ca^{2+} effects are half-maximal at a Ca^{2+} concentration of $2 \cdot 10^{-7}$ M. They can be explained by assuming that ATPase activity is optimal in a medium range of actin polymer stability and that micromolar Ca^{2+} concentrations tend to labilize and depolymerize F-actin.

Introduction

We have recently described (Löw and Dancker [1]) that the action of the mold metabolite cytochalasin B on actin can be intensified by the Ca^{2+} chelating agent EGTA. This observation means that micromolar concentrations of Ca^{2+} (which are present in all assays without EGTA) prevent the full action of cytochalasin B. This observation stimulated our interest for two reasons: (1) Up to the present effects of micromolar Ca^{2+} concentrations are well documented only for actin filaments containing the regulatory proteins tropomyosin and troponin (for review see Ebashi and Endo [2], Weber and Murray [3]) but not for actin filaments devoid of these proteins. (2) When actin is able to respond to changes of Ca^{2+} concentrations in the micromolar range it is conceivable that cells utilize this property for the control of the polymer state of their actin.

In this paper we describe effects of Ca^{2+} on the ATPase (ATP phosphohy-

Abbreviations: EGTA: ethyleneglycol-bis(2-aminoethylether)-*N,N'*-tetraacetic acid. Tris: Tris · HCl buffer. His: histidine-HCl buffer. SDS: sodium dodecyl sulfate.

drolase, 3.6.1.3) activity of actin induced by sonication (cf. Asakura [4]) rather than effects on cytochalasin B-induced ATPase activity because in the case of sonication we can be sure that Ca^{2+} effects are due to alterations in actin itself and not due to possible interferences of Ca^{2+} with the binding of cytochalasin B to actin. The main result of our study is that there are two opposing actions of Ca^{2+} on actin: one exerted by micromolar concentrations of Ca^{2+} (destabilizing and/or depolymerizing) and one exerted by millimolar concentrations (polymerizing).

Experimental

The preparation of actin (yielding only one band in SDS polyacryl amide gel electrophoresis indicating that there are no other proteins present) from rabbit skeletal muscle, the measurement of ATPase activity etc. were performed as described earlier [1,5]. Depolymerized actin was obtained in the following way: F-actin pellets were extensively homogenized with a teflon homogenizer at 0° C in 0.1 mM ATP, 1 mM Tris · HCl buffer, pH 8.0. The homogenized actin was then dialyzed against a solution with the same composition for 48 h at 4° C and thereafter homogenized once more. Further details are indicated in the figures. For adjusting the desired low Ca^{2+} concentrations the assays contained 1 mM EGTA and varying concentrations of $CaCl_2$. The values of free Ca^{2+} indicated in Fig. 1 are based on the assumption that under our conditions (which were often those of very low ionic strength) the stability constant of the Ca · EGTA complex was $5.17 \cdot 10^6\ M^{-1}$, cf. Schwarzenbach [6]. Since we deduced an affinity of Ca^{2+} to actin which was equal in the presence as well as in the absence of 90 mM KCl (see below) we take this as evidence that the variation of ionic strength did not introduce a big error. Ca · EGTA was neutralized with imidazole, the pH of the experimental assays was, if not otherwise indicated, adjusted with histidine buffer to pH 7.0.

Results

Fig. 1 A and B show the effect of micromolar Ca^{2+} concentrations on the ATPase activity of F-actin induced by sonication. Increasing concentrations of Ca^{2+} reduced the ATPase activity of actin when KCl was absent. In the presence of KCl, however, ATPase activity was enhanced rather than reduced by micromolar concentrations of Ca^{2+}. The reducing (though not fully inhibiting) as well as the activating effect of Ca^{2+} could be described by assuming a hyperbolic relationship between the concentration of free Ca^{2+} and the Ca^{2+}-sensitive part of the ATPase activity with a half-maximal effect of Ca^{2+} at a concentration of free Ca^{2+} of $2 \cdot 10^{-7}$ M. This effect is in fact due to Ca^{2+} and not due to EGTA since the concentration of EGTA remained constant in our Ca^{2+} buffers.

Ultrasonic ATPase activity is a property of actin filaments. ATPase activity can therefore be influenced either by changing the fraction of F-actin or by influencing the stability of existing filaments (the latter explains the inhibition of ultrasonic ATPase activity by the mushroom poison phalloidin, cf. Dancker et al. [5]). In order to evaluate these two alternatives we compared the

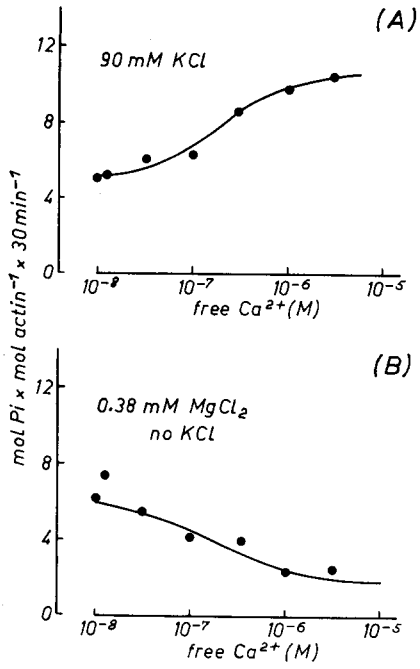

Fig. 1. Effect of free Ca^{2+} concentration on ATPase activity of actin. Depolymerized actin was repolymerized for 24 h in 1 mM histidine buffer, pH 7.0, 0.1 mM ATP and either 0.1 M KCl (A) or 0.45 mM $MgCl_2$ (B) at 4°C. The hydrolysis of ATP was measured in 2-ml assays containing 1.6 mg actin (actin concentration 18 μM), 90 mM KCl (A) or 0.38 mM $MgCl_2$ (B), 1 mM ATP and 10 mM histidine buffer, pH 7.0. The free Ca^{2+} concentration indicated at the abscissas was adjusted with CaEGTA buffers (containing 1 mM EGTA and varying amounts of $CaCl_2$). The values of P_i indicated at the oridinate are derived from the difference in P_i content of the sonicated samples and samples without sonication to which immediately after the addition of ATP trichloroacetic acid was added. The lines are adsorption isotherms and are calculated on the basis of the assumption that there exists a hyperbolic relationship between the concentration of free Ca^{2+} and the part of the ATPase activity which varies with Ca^{2+} concentration and that the half-maximal Ca^{2+} effect is obtained at a concentration of free Ca^{2+} of $2 \cdot 10^{-7}$ M.

influence of Ca^{2+} on both ultrasonic ATPase activity and on actin polymerization (measured by means of viscosity) at different concentrations of $MgCl_2$ (Fig. 2). This figure shows that the removal or addition of Ca^{2+} influenced actin polymerization and ATPase activity in an equal manner under appropriate conditions. Removal of Ca^{2+} by EGTA stimulated ATPase activity as well as actin polymerization at low concentrations of Mg^{2+} (cf. also table). From these results it can be inferred that micromolar concentrations of Ca^{2+} depress actin polymerization as they depress tubulin polymerization (cf. Weisenberg [7], Haga et al. [8], Hayashi and Matsumura [9]; according to Olmstedt and Borisy [10] Ca^{2+} inhibits tubulin polymerization in the millimolar range). It can be further inferred that Ca^{2+} acts on ultrasonic ATPase activity by interfering with the polymerization of actin.

The depression of actin polymerization by micromolar concentrations of Ca^{2+} is in contrast to the well-known fact that millimolar concentrations of Ca^{2+} stimulate actin polymerization in quite a similar manner as Mg^{2+} ions do but is in line with the observation reported by Isenberg and Wohlfarth-Bottermann [11] that EGTA is able to stimulate polymerization of actin from the

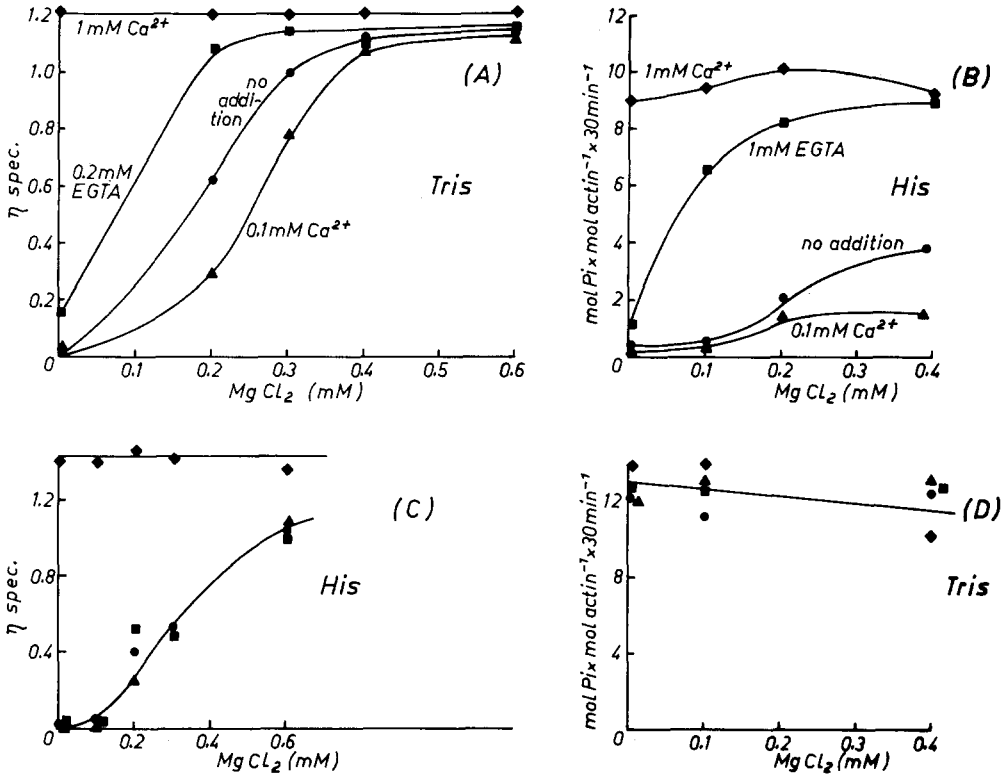

Fig. 2. The effect of Ca^{2+} on polymerization and ATPase activity of actin at different concentrations of $MgCl_2$. Polymerization (A, C) F-actin pellets were depolymerized and then divided into different portions containing 1.2 mg actin/ml, to which $MgCl_2$ and $CaCl_2$ or EGTA were added so that the concentrations of Mg^{2+}, Ca^{2+} and EGTA as indicated in the figures were obtained. In the case of (A) the samples contained 5 mM Tris/maleate buffer, pH 7.2, in the case of (C) 5 mM histidine buffer, pH 7.0 Each sample contained 0.1 mM ATP. These samples were allowed to stand for 15 h at 4° C, thereafter viscosity was measured at 23° C. η spec. denotes specific viscosity. ATPase activity during sonication at 20° C ((B), (D)): 2 ml samples of depolymerized actin, containing 1.6 mg actin in 10 mM histidine buffer, pH 7.0 (B) or in 10 mM tris buffer, pH 7.4 (D), 1 mM ATP and different concentrations of Mg^{2+} as indicated at the abscissae, were sonicated at 20° C in the presence or absence of 1 mM EGTA, 0.1 or 1.0 mM Ca^{2+}. The reference samples were not sonicated but stopped immediately after the addition of 1 mM ATP with trichloroacetic acid. The actin concentration was 18 μM. The meaning of the symbols in (C) and (D) is the same as in (A) and (B).

slime mold *Physarum polycephalum* and is further in line with the experience of Kane [12] that for full "gelation" of actin from sea-urchin eggs EGTA must be present.

Accordingly, a dual behavior of Ca^{2+} is to be expected and this in fact is found in Fig. 2: addition of Ca^{2+} to a concentration of 0.1 mM depressed actin polymerization as well as ATPase activity whereas further addition of Ca^{2+} to 1 mM activated polymerization as well as ATPase activity to such an extent that they both became independent of Mg^{2+}.

The results reported so far have shown that the action of Ca^{2+} depends on other factors (e.g Mg^{2+}, K^+) which affect actin polymerization. One of these other factors affecting actin polymerization and therefore Ca^{2+} action is the kind of buffer ions used. This can also be seen from Fig. 2. The activating effect of Ca^{2+}

removal on ultrasonic ATPase activity could clearly be observed only in the presence of histidine buffer but not in the presence of Tris buffer (Tris · HCl, pH 7.4 or Tris/maleate, pH 7.2). This explains why Dancker et al. [5] who measured ultrasonic ATPase activity in the presence of 5 mM Tris · HCl did not see an effect of EGTA. In 10 mM Tris · buffer ATPase activity was already maximal without addition of $MgCl_2$ or EGTA (Fig. 2 D), so that no further activating effect of EGTA could be expected. The low ATPase activity at low concentrations of Mg^{2+} in the presence of histidine buffer (as compared to that in the presence of Tris, cf. Fig. 2 B and D) is no effect of pH 7.0 (pH 7.0 cannot be attained with Tris) but a property of histidine itself because at pH 7.0 high ATPase activity is possible without addition of $MgCl_2$ when histidine buffer is replaced by imidazole buffer. We give the following example: Without added $MgCl_2$ there were 7.1 mol ATP · mol actin^{-1} · 30 min^{-1} hydrolysed in the presence of imidazole whereas in the presence of histidine buffer the respective value was 0.5.

In the case of actin polymerization the relation between Ca^{2+} effects and buffers used was just opposite to that in the case of ATPase activity: Ca^{2+} acted more efficiently in the presence of Tris buffer (Fig. 2 A) than in the presence of histidine buffer (Fig. 2 C). This apparent discrepancy can be explained in the following way: the Tris cation itself stimulates actin polymerization more efficiently than histidine buffer does (less $MgCl_2$ is needed to attain maximal polymerization in the presence of Tris as compared to the presence of histidine). Therefore when present at a concentration of 10 mM, Tris together with the stimulating effect of sonication, provides a polymerizing milieu which generates sufficient filaments for maximal ATPase activity even without added $MgCl_2$ and without Ca^{2+} removal. (The polymerizing effect of sonication is discussed by Asakura et al. [13] and Nakaoka and Kasai [14] and is obviously related to the fact that sonication increases the number of filament endings by disintegrating existing filaments). At 5 mM the stimulating action of Tris is weaker and therefore a Ca^{2+} effect is visible (see Table I). That, on the other hand, the Ca^{2+} effect on polymerization without sonication (that is the Ca^{2+} effect, measured by means of viscosity) was nearly absent in the presence of histidine, is probably due to the fact that, in the presence of histidine, too much $MgCl_2$ is needed for polymerization to occur (a certain amount of filaments is necessary for the Ca^{2+} effects to become visible) and that these higher Mg^{2+} concentrations compete with Ca^{2+} for the micromolar binding sites.

TABLE I

INFLUENCE OF EGTA ON ATP HYDROLYSIS BY F-ACTIN INDUCED BY SONIC VIBRATION. DEPENDENCE ON Mg^{2+} CONCENTRATION

ATPase activity: mol P_i · mol actin^{-1} · 30 min^{-1} at 20°C. Actin concentration: 12 μM in 5 mM Tris buffer, pH 7.4; EGTA was, if present 0.2 mM.

	−EGTA	+EGTA
90 mM KCl	5.7	3.7
0.175 mM $MgCl_2$	4.0	8.8
0.35 mM $MgCl_2$	5.5	8.6
0.70 mM $MgCl_2$	6.7	6.9

Discussion

In this contribution we document a dual effect of Ca^{2+} on the structural behavior of actin. Besides the well-known polymerizing effect of Ca^{2+} at millimolar concentratins we report a labilizing effect of Ca^{2+} in the micromolar range. We have described that (1) micromolar concentrations of Ca^{2+} depress at low concentrations of Mg^{2+} the ultrasonic ATPase activity of F-actin, probably by reducing the amount of ATP-splitting filaments, that (2) micromolar Ca^{2+} concentrations activate rather than reduce ultrasonic ATPase activity in the presence of KCl and (3) these Ca^{2+} effects are half-maximal at a concentration of free Ca^{2+} of $2 \cdot 10^{-7}$ M. Results (1) and (2) can be explained with the aid of the following model: It is assumed that actin can change its structure from G-actin via flexible polymers to rigid filaments (with low flexibility) and vice versa (Fig. 3). Increasing concentrations of Mg^{2+}, Ca^{2+} (beginning with 0.1 mM), KCl (and phalloidin, cf. Dancker et al. [5]) tend to shift the structure of actin to the right. Micromolar concentrations of Ca^{2+}, on the other hand, tend to shift actin structure to the left. During sonication the medium range of stability, where according to this model flexible polymers exist, is adopted. It is further assumed that ATPase activity of actin is optimal in the medium range of flexible polymers. When actin structure lies at the right-hand side of the activity optimum (as it is assumed for the presence of KCl) the shift to the left performed by micromolar Ca^{2+} concentrations will activate ATPase activity. When actin structure lies at the left-hand side of the activity optimum, micro-

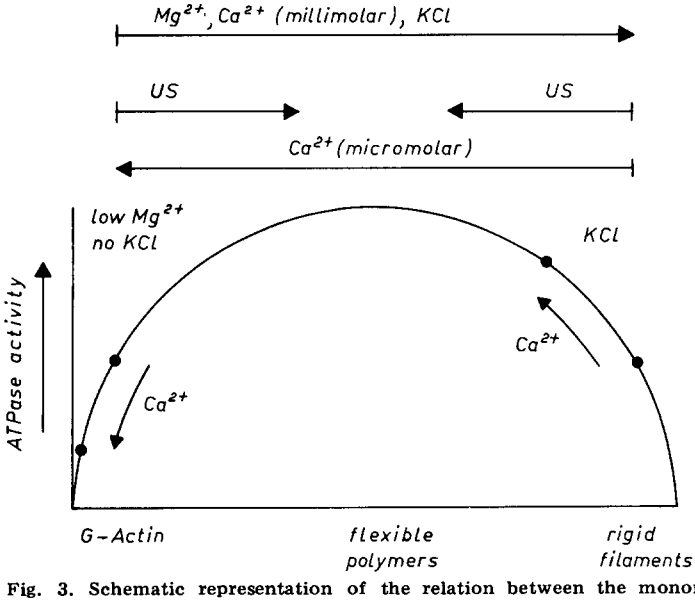

Fig. 3. Schematic representation of the relation between the monomer-polymer structure of actin (abscissa) and ATPase activity of actin (ordinate) as it is deduced from our experiments. The arrows on top of the diagram represent the direction into which the parameters indicated tend to shift actin structure. Sonication (US, from ultrasound) is assumed to favor the medium range of stability in which flexible polymers exist by either tending to loosen rigid filaments or by stimulating polymerization (when sonication is applied to weakly polymerized actin).

molar concentrations of Ca^{2+} will depress ATPase activity. In the medium range of flexible polymers there should be only a small Ca^{2+} effect (or even no effect at all). A Ca^{2+} effect was absent in the presence of 10 mM tris buffer during sonication (Fig. 2 D), therefore one can assume that under those conditions actin was in the medium range of flexible polymers. Hence the action of Ca^{2+} depends on the whole set of conditions which influence actin structure. This may explain why Bárány and Finkelman [15], sonicating actin under rather stabilizing conditions (relatively high concentrations of actin, presence of KCl) have not observed an influence of EGTA on ultrasonic ATPase activity.

The result (3) mentioned above (concerning the apparent affinity of Ca^{2+} to actin) deserves special evaluation because it shows that pure actin (devoid of tropomyosin-troponin) is able to respond to Ca^{2+} in the micromolar range. Consequently the actin unit itself must possess at least two binding sites for Ca^{2+}: one which is saturated at micromolar concentrations of Ca^{2+} and which labilizes the polymer structure of actin and one which is saturated at millimolar Ca^{2+} concentrations and which leads to polymerization. The "micromolar" binding site must probably be distinguished from the site which is involved in nucleotide binding of G-actin. The Ca^{2+} affinity for this latter site is in the range of 10^5 M^{-1} (Waechter and Engel [16], Waechter [17]). A similar Ca^{2+} affinity to F-actin during sonication is reported by Kasai and Oosawa [18], whereas we deduced for the micromolar binding site an affinity constant of $5 \cdot 10^6$ M^{-1} (see Fig. 1).

The "micromolar" Ca^{2+}-binding site may well be a control site since it binds Ca^{2+} in a concentration range which can be controlled by the cell. The control of actin stability in non-muscle cells is a matter of considerable interest (see Isenberg and Wohlfarth-Bottermann [11], Tilney [19], Pollard [20]). In this context it should mentioned that a labilizing action of increasing intracellular Ca^{2+} concentrations on actin filaments has been inferred from the results of Poste et al. [21,22]. These workers have observed that certain local anaesthetics disrupt not only microtubuli but also actin-like microfilaments of fibroblasts. This has been interpreted as being due to displacement of Ca^{2+} from intracellular binding sites by these local anaesthetics making Ca^{2+} available for binding to both microtubuli and microfilaments. Hence a possible regulatory role of Ca^{2+} for the filament structure of actin should be seriously considered.

Acknowledgement

We thank M. Hoffmann for valuable help and Professors W. Hasselbach and Th. Wieland for their interest and for reading the manuscript.

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