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GELSOLIN IS Ca^{2+}-SENSITIVE REGULATOR OF ACTOMYOSIN SYSTEM IN PLATELET

Toyoshi Onji*, Michiko Takagi and Nobuhiko Shibata

Division of Molecular Cardiology
The Center for Adult Diseases, Osaka
1-3-3, Nakamichi, Higashinariku, Osaka
537, Japan

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Effects of gelsolin on the actomyosin system in platelet have been studied. MgATPase activity of platelet actomyosin is enhanced up to two folds by 200 nM of platelet gelsolin in the presence, but not in the absence of Ca ion. The half maximum enhancement is observed at the concentration of Ca^{2+} around 10^{-5} M . The effect of gelsolin to enhance the ATPase activity of actomyosin is potentiated by tropomyosin, which is a Ca^{2+}-insensitive actomyosin enhancer. The results indicate that gelsolin may control the activity of actomyosin system in platelets.

Actomyosin system is the final effector of platelets in response to activating stimuli. Activity of the system is controlled by phosphorylation of myosin and reorganization of actin. The former mechanism is rather straightforward(1,2,3), while the latter mechanism has remained unclear. The reason for the poor characterization of actin reorganization is that many factors have been found to interact with actin,in vitro(4). One of the most enigmatic actin-associated proteins is gelsolin,which was found in macrophage by Yin and Stossel(5). The protein was later reported to exist in platelet by the same group(6). The function of gelsolin in platelets,however, is difficult to understand because of the following reason. The remarkable

*To whom correspondence should be addressed.

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activity of gelsolin to sever actin filament in vitro contradicts the distinct bundling of actin which is observed after the activation of platelets. This contradiction can be solved only if the effect of gelsolin to sever F-actin is shown to be non-physiological,or severing effect is manifested in very localized time and space. If the severing effect is non-physiological,the true effect of gelsolin in platelets should be determined. The studies of gelsolin,however,have been limited to actin-gelsolin interaction. Cellular actin should not be regarded as an isolated system. It should be regarded as a component of actomyosin motile system. The effect of gelsolin on this system should be examined before its physiological roles are determined. We have reported effects of several actin-binding proteins(tropomyosin, filamin, and caldesmon) on the actomyosin system in platelet(7,8,9,10,11). In this report:(i)we describe a method to purify gelsolin, and alpha-actinin from same porcine platelet source, and (ii)it is shown that platelet gelsolin acts as a Ca^{2+}-sensitive regulator of actomyosin ATPase activity.

MATERIALS AND METHODS

(1)Purification of proteins.

Porcine platelets were homogenized and the 100,000 g supernatant was applied to DEAE-Sephacel anion-exchange resin(Pharmacia LKB) and was eluted by linear KCl gradient as described by Gordon et al.(12). Filamin and actomyosin (Fig.1:(a)-1) were purified from the fractions at the concentration of KCl of 0.16-0.20M and 0.21-0.25M,respectively(9). Actin (Fig.1:(a)-3) was purified from the fractions at the concentration of KCl of 0.25-0.3M. Gelsolin and alpha-actinin were found at the concentration of KCl of 0.28-0.31M.These fractions were combined and condensed by (NH4)2SO4 at the concentration from 30 to 40%.The protein was dialyzed against 0.1M KCl,2mM EDTA,1mM dithiothreitol,and 10mM imidazole-HCl(pH 7.0) and was subjected to high performance liquid chromatography separation on a QAE column(Pharmacia LKB) . Gelsolin(Fig.1:(a)-5) was eluted at the concentration of KCl of 0.24M, while alpha-actinin(Fig.1:(a)-4) was eluted at the concentration of KCl of 0.37M.

Platelet tropomyosin(Fig.1:(a)-7) was purified as described previously(7).

(2)Assay methods.
ATPase activity of platelet actomyosin was measured as previously(7-11). Viscosity of platelet actin was measured as previously(9,10). Assay conditions are stated in the figure legends.

(3)Other methods.
Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed by the method of Laemmli(13). Protein concentration was determined by the method of Bradford(14),using bovine serum albumin as a standard.

RESULTS

(1) Purification and properties of platelet gelsolin and alpha-actinin.

Previous studies utilized DNaseI-agarose affinity chromatography and other routine chromatographic methods to isolate gelsolin(5,6,15,16). Utilization of high-performance liquid chromatography has advantage over the previous techniques as follows:(i)actin,actomyosin,filamin,gelsolin and alpha-actinin are all purified after DEAE-Sephacel anion-exchange chromatography, (ii) these actin-associated proteins are free from contamination of actin,which was the problem inherent in the previous methods.

Platelet gelsolin (molecular weight around 95,000) purified by this method decreases the viscosity of platelet actin markedly only in the presence of Ca ion (over 10^{-6}M)(Fig.1(b): solid line with open circle).Platelet alpha-actinin (molecular weight around 105,000) increases the viscosity of platelet actin (Fig.1(b): broken line with closed circle). The effect of alpha-actinin is abolished by gelsolin in the presence of Ca ion(Fig.1(b):broken line with closed circle).The effect is not affected by gelsolin in the absence of Ca ion (data not shown).

(2)Effects of platelet gelsolin on the actomyosin ATPase activity.

The effect of gelsolin on the ATPase activity of platelet actomyosin was then examined. As shown in Fig.2,MgATPase activity

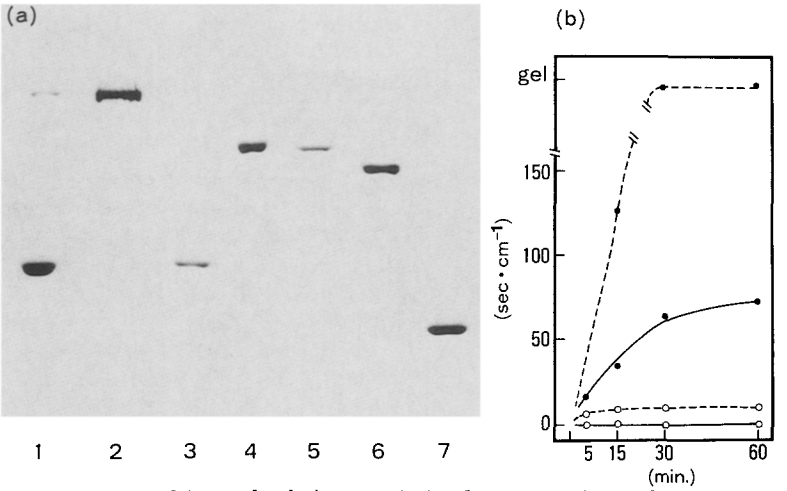

Figure 1:Gelsolin and alpha-actinin from porcine platelets.

(a)SDS-polyacrylamide gel electrophoretogram of contractile proteins purified from porcine platelets.

1,platelet actomyosin;2,platelet myosin; 3,platelet actin; 4,platelet alpha-actinin;5,platelet gelsolin;6,platelet caldesmon; 7,platelet tropomyosin.

(b)Effects of platelet gelsolin and/or alpha-actinin on the viscosity of platelet actin.

Time course of the changes in the viscosity of platelet actin in the presence or absence of platelet gelsolin and/or platelet alpha-actinin is shown .

Assay conditions:(1)Protein concentrations;[platelet actin]= 1.5×10^{-5}M,[platelet gelsolin]=1.0×10^{-8}M, [platelet alpha-actinin] =1.0×10^{-6}M. (2)Reaction medium;[KCl]=100mM,[MgCl2]=2mM, [CaCl2] (open circle)=100nM, [EGTA](closed circle)=2mM,[imidazole-HCl]= 10mM,pH 7.0.

Solid line with closed circle;control(platelet actin alone:addition of gelsolon has no effect in this condition).
Solid line with open circle;platelet actin plus platelet gelsolin.
Broken line with closed circle;platelet actin plus platelet alpha-actinin(addition of gelsolin has no effect in this condition).
Broken line with open circle;platelet actin plus platelet gelsolin and platelet alpha-actinin.

of platelet actomyosin is enhanced markedly in the presence of platelet gelsolin. The concentration of platelet gelsolin required to enhance the activity half-maximally is around 10^{-7}M. The value is slightly higher than its concentration required to decrease the viscosity of F-actin half-maximally. It is noted that the enhancement is observed only in the presence of Ca ion. If Ca ion is not present, gelsolin slightly inhibits the MgATPase activity of platelet actomyosin (Fig.2: dotted line).

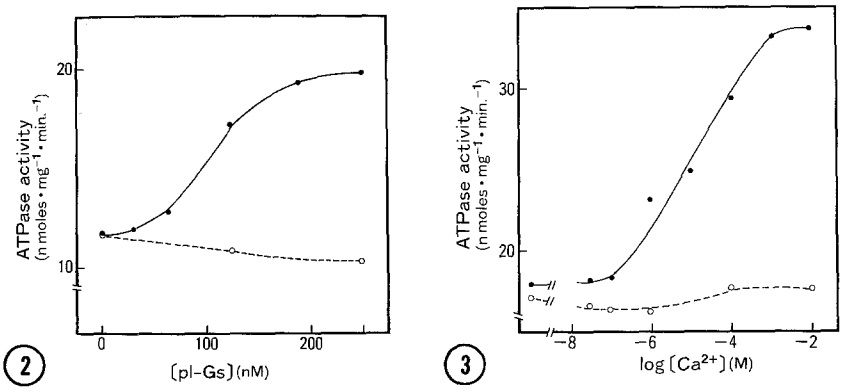

Figure 2:Effects of platelet gelsolin on the MgATPase activity of platelet actomyosin.

The effect of platelet gelsolin on the MgATPase activity of platelet actomyosin in the presence(solid line) or absence (broken line) of Ca^{2+} was measured.

Assay conditions:(1)Protein concentrations;[platelet actomyosin]=1.0x10^{-4}g/ml,[platelet gelsolin]=0-250nM, (2)Reaction medium;[KCl]=100mM, [MgCl2]=5mM, [CaCl2](solid line)=0.1mM, [EGTA](broken line)=2mM,[imidazole-HCl]=10mM,pH7.0. Other conditions are the same as before and are stated in our previous reports(7-11).

pl-Gs:platelet gelsolin

Figure 3:Dependency of the effect of gelsolin on the concentration of Ca ion.

The MgATPase activity of platelet actomyosin in the presence or the absence of platelet gelsolin was measured at increasing concentrations of Ca^{2+}. Assay condition was the same as in Fig.2,except that the concentration of platelet gelsolin was fixed(200nM) and the concentration of Ca^{2+} was varied using Ca-EGTA buffer system (22).

Solid line;in the presence of platelet gelsolin.
Broken line;in the absence of platelet gelsolin.

The dependency of the effect of gelsolin on the concentration of Ca ion is depicted in Fig.3. The MgATPase activity of purified actomyosin is not controlled by the concentration of Ca ion(broken line).However,if platelet gelsolin is added to this system, the MgATPase activity of platelet actomyosin becomes strictly dependent upon the concentration of Ca ion. The ATPase activity is enhanced as the concentration of Ca ion is increased, and it levels off at the concentration of Ca ion of 10^{-3}M. Half maximum enhancement is observed at its concentration around 10^{-5}M,which is higher than the concentration to manifest the activity of gelsolin to sever F-actin.

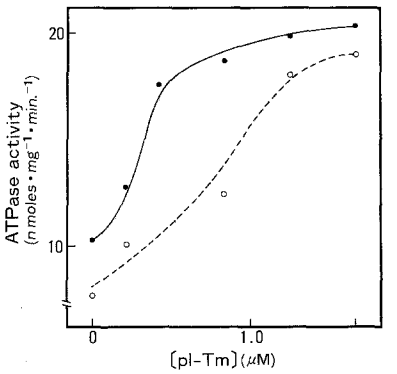

Figure 4:Effect of gelsolin in the presence of tropomyosin.

The effect of platelet gelsolin on the MgATPase activity of platelet actomyosin was measured in the presence of increasing concentrations of platelet tropomyosin.
Assay conditions are the same as in Fig.2, except that the concentration of platelet gelsolin was fixed (100nM).

Solid line;in the presence of platelet gelsolin.
Broken line;in the absence of platelet gelsolin.

pl-Tm:platelet tropomyosin.

Previously, we reported that tropomyosin is an important factor to determine the state of actomyosin in platelets. It enhances MgATPase activity of actomyosin(7),and the effect itself is controlled by platelet caldesmon(11). It is of interest to see if the effect of gelsolin to enhance the MgATPase activity is modified by tropomyosin. The results of such an experiment are shown in Fig.4.

As we reported previously, platelet tropomyosin enhances MgATPase activity of platelet actomyosin. This effect is not affected by Ca ion. When gelsolin is added to actomyosin-tropomyosin system, the effect of gelsolin and tropomyosin to enhance the activity are synergistically increased(solid line in Fig.4). It appeared that the effect of tropomyosin at the non-saturating concentration is enhanced much by platelet gelsolin. It should be noted that gelsolin does not affect the tropomyosin-actomyosin system if Ca ion is not present (identical with the broken line in Fig.4).

DISCUSSION

We have elucidated that many actin-associated proteins are capable of affecting actomyosin system in platelet. Some of these effects are mediated through the changes in the state of actin filaments. Thus , the effect of actin cross-linking proteins (e.g.filamin) is usually inhibitory to actomyosin ATPase activity(9,10).However, the effects of other actin associated proteins (e.g. caldesmon) is probably exerted at the molecular level. The effect of tropomyosin may be exerted at both levels. The mechanism by which platelet gelsolin enhances actomyosin ATPase activity is not clear. It is possible that fragmentation of F-actin is favorable to actin-myosin interaction ,because cytochalasin B which is known to sever F-actin can enhance the ATPase activity of platelet actomyosin (unpublished observation). The effect is not completely explained by its ability to sever F-actin, because tropomyosin, which is shown to inhibit fragmentation of F-actin by gelsolin,does not inhibit,but potentiates the effect of gelsolin to enhance the ATPase activity.

Although molecular properties of gelsolin have been described in detail (16,17), its physiological function remains puzzling. The most remarkable property of gelsolin to sever actin filaments seems to contradict the bundling of actin observed after the activation of platelets. This difficulty was alleviated by a recent finding that phosphatidylinositol 4,5-bisphosphate , which is produced as a result of the increased turnover of phosphatidylinositol, inhibits the ability of gelsolin to sever actin filaments. Its function to nucleate actin assembly, however, is not affected(19). This result is compatible with a view that gelsolin acts as a promoter, not as an inhibitor of actin polymerization. The difficulty in this view is that

the most interesting property of gelsolin, namely, Ca ion dependency of its effect, becomes unexplainable if its function is controlled by phosphatidylinositol 4,5-bisphosphate, and not by Ca ion.

Previous studies on gelsolin in non-muscle cells were limited to its interaction with F-actin. If the enhancement of actomyosin ATPase activity by gelsolin is physiological,gelsolin can be regarded as an enhancer of actomyosin system. The enhancement can be physiological, because the function is controlled by Ca ion concentration at the physiological range.The view that gelsolin could acts as an enhancer of actomyosin system is supported by the fact that gelsolin is synergistic with tropomyosin , which is a known enhancer of platelet actomyosin(7,8) and an inhibitor of the severing function of gelsolin (20). Another evidence to support the view is that gelsolin is known to inhibit the effect of filamin,a cross-linker of F-actin(21), which interferes with actin-myosin interaction(9,10).

We postulate that the effect of gelsolin on the actomyosin-tropomyosin system is enhancing, not inhibiting. The effect of gelsolin to sever F-actin in the presence of Ca ion is manifested only under limited conditions, where myosin and tropomyosin are not bound to F-actin and when the influence of phosphatidylinositol-4,5 bisphosphate is negligible. Only under such conditions could gelsolin serve as a regulator of gel-sol transition of cytoplasm.

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