A SPECIFIC 1:1 G-ACTIN:DNAase I COMPLEX FORMED BY THE ACTION OF DNAase I ON F-ACTIN

H. G. MANNHERZ, J. BARRINGTON LEIGH, R. LEBERMAN and H. PFRANG
Max-Planck-Institut für Medizinische Forschung, Abteilung Biophysik, 69 Heidelberg, Jahnstraße 29, West Germany

Received 8 September 1975

1. Introduction

In 1967 Lindberg [1] reported the isolation of a protein from calf spleen which forms a 1:1 complex with DNAase I thereby inhibiting its activity. Quite recently Lazarides and Lindberg [2] have shown that G-actin inhibits DNAase I specifically and that this inhibition may be a natural role of actin. Furthermore, Lazarides and Lindberg [2] demonstrated that this DNAase inhibitor and G-actin share a number physicochemical properties, e.g. comigration on polyacrylamide gels in the presence of SDS, similar amino acid composition and tryptic peptide maps, and the ability to form high molecular weight polymers. It was therefore thought to be of interest to examine whether DNAase I would effect the polymerization of G-actin and conversely whether polymerized actin (F-actin) would interact with DNAase I, since actin is found in the filamentous form in situations involving directed motion. Furthermore, it is important to examine the reversibility of the DNAase I–actin interaction.

2. Materials and methods

Actin was prepared from rabbit psoas muscle acetone dried powder according to A. Szent-Györgyi [3] and taken up in a solution containing 5 mM HEPES-buffer pH 7.5, 0.1 mM $CaCl_2$, 0.1 mM NaN_3, and 0.2 mM ATP. DNAase I was obtained from Worthington Corp. USA, and was found on polyacrylamide gels in the presence of SDS to be about 60–70% pure. In those experiments where the enzyme was used as supplied care was taken to inhibit any contaminating proteases by addition of 10^{-4} M PMSF.

In most experiments, however, DNAase I was further purified on hydroxylapatite as discribed by Price et al. [4] yielding an enzyme of single band purity on polyacrylamide gels in the presence of SDS (mol. wt, approx. 33 000).

Myosin subfragment-1 was prepared according to Lowey et al. [5].

Viscosity measurements were carried out using an Ostwald type viscometer giving a water out flow time of 37 sec at 25°C. Gel-electrophoresis on polyacrylamide gels in the presence of SDS was performed according to Ziegler et al. [7].

ATPase activity was measured using an Aminco spectrophotometer using the optical method as described by Trentham et al. [8]. Fluorescence of ϵATP–G-actin ($1,N^6$-ethenoadenosine triphosphate) was measured at 410 nm in a Perkin-Elmer MPF-3 Fluorospectrophotometer exciting at 340 nm. ϵATP was a generous gift of Dr R. S. Goody and ϵATP–G-actin was prepared by Dr E. Nowak. Tropomyosin was prepared according to Bailey [6] and was a generous gift of Dr E. Nowak.

3. Results

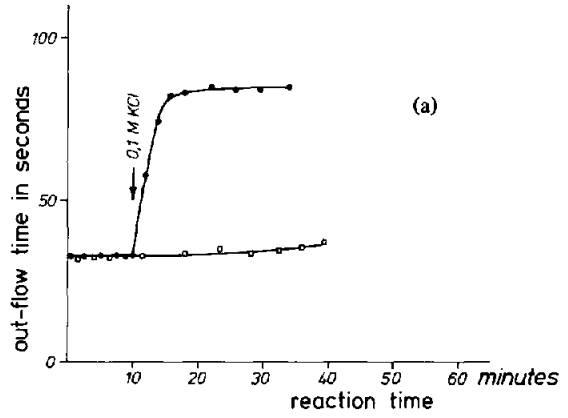
3.1. *Inhibition of polymerization of G-actin and depolymerization of F-actin by DNAase I*

On addition of mono- or divalent salts G-actin

Abbreviations: HMM-S1, Myosin subfragment 1; ATP, adenosine triphosphate; ϵATP, 1, *N*-6-ethenoadenosine triphosphate; DNAase I, Deoxyribonuclease I; HEPES, 2-[4-(2-hydroxethyl)-piperazine-(1)]-ethanesulfonic acid; SDS, sodium dodecylsulfate; PMSF, phenylmethylsulfonylfluoride.

North-Holland Publishing Company – Amsterdam

forms filamentous polymers closely resembling thin filaments of muscle. This process can be followed by observing the increase in viscosity, as shown in fig.1(a) and is complete after about 5 to 10 min. Preincubation of G-actin with an equimolar amount of DNAase I inhibits completely polymerization of G-actin after addition of 0.1 M KCl as judged by viscometric measurements. Therefore the converse experiment starting with various F-actin preparations was carried out and in illustrated in fig.1(b). G-actin was polymerized by addition of 0.1 M KCl as pure G-actin, in the presence of one seventh molar equivalent tropomyosin or nearly one molar equivalent of HMM-S1. After formation of the actin polymer two different amounts of DNAase I were added to pure F-actin (final concentrations : actin 2 mg/ml; DNAase I 0.33 mg/ml or 2 mg/ml). This resulted in a marked drop in viscosity the reaction being complete in about 30 min. Addition of DNAase I to F-actin at a lower than 1:1 molar ratio produces only an incomplete drop in viscosity, whereas at about equimolar ratio pure F-actin is almost completely depolymerized. The same result is obtained for actin polymers containing tropomyosin or HMM-S1 without any significant difference in rate and extent of depolymerization by DNAase I. In a different experiment starting with 1 mg/ml G-actin and one seventh molar equivalent of tropomyosin, the polymer formed depolymerized after addition of one molar equivalent of DNAase I just as well. Then 4.7 mg HMM-S1 (giving two molar excess of HMM-S1 over actin monomer concentration) were added and a slow



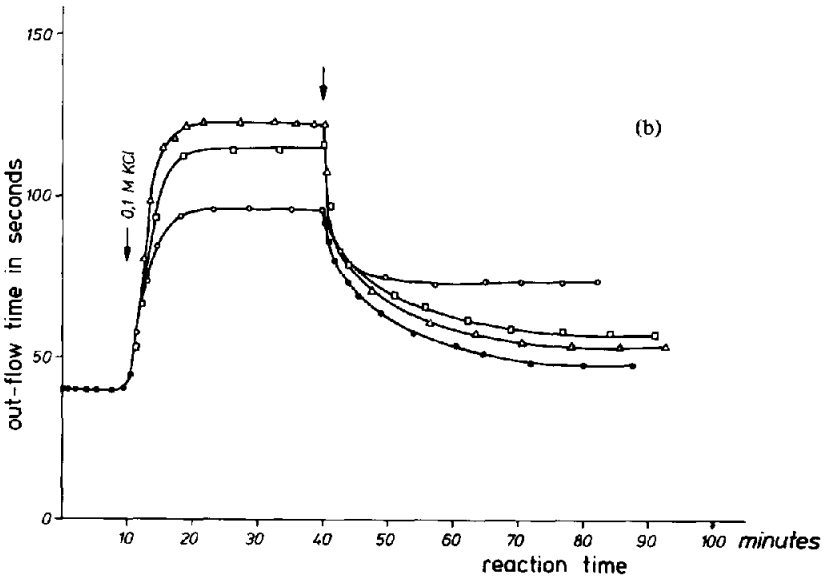


Fig.1(a) Effect of addition of 0.1 M KCl on the relative viscosity to a solution of actin (2 mg/ml) (•——•) and to a solution of actin (2 mg/ml) and DNAase I (2 mg/ml) □——□. Abscissa: time in minutes, ordinate: measured out-flow time, in seconds. (b) After polymerization (first arrow) with 0.1 M KCl the drop in relative viscosity after DNAase I addition was measured against time (second arrow) for: (•——•) pure actin (2 mg/ml) added DNAase I (2 mg/ml); (○——○) pure actin (2 mg/ml) added 0.33 mg/ml DNAase I; (□——□) actin (2 mg/ml) and tropomyosin (0.5 mg/ml) added 2 mg/ml DNAase I; (△——△) 1.1 mg/ml actin plus 2.6 mg/ml HMM-S1 added 1.5 mg/ml DNAase I.

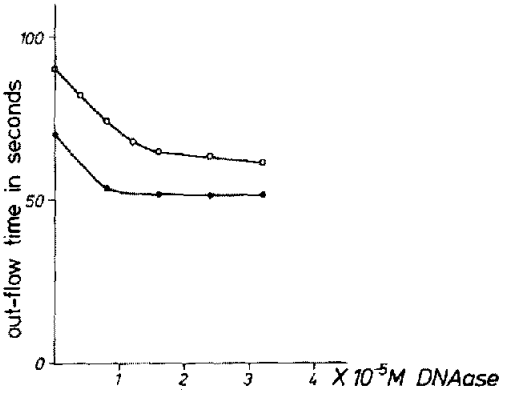


Fig.2. Effect of increasing concentration of DNAase I on the relative viscosity of F-actin. (●——●) $3 \cdot 10^{-5}$ M F-actin, (○——○) $6 \cdot 10^{-5}$ M F-actin. Abscissa: final concentration of DNAase I added, ordinate: measured out-flow time in seconds.

increase in viscosity was observed (completed in about two hours) showing a 50% higher viscosity than the starting value. This result indicates that the DNAase I–actin complex is dissociated by the concerted action of tropomyosin and HMM-S1. This point, however, needs further investigation as does the possible protecting effect of troponin.

As the extent of depolymerization of F-actin appeared to be dependent on the concentration of DNAase I, F-actin solutions of two different concentrations were titrated with DNAase I and the reduction in relative viscosity was measured (fig.2). It can be seen that the viscosity is reduced to the plateau value already when a molar ratio of DNAase I to actin of 0.27 was reached. This may indicate that the F-actin polymer is broken up into smaller segments by DNAase I, which causes the drop of viscosity to the initial value to occur at less than equimolar concentrations of DNAase I to actin.

3.2. *Inhibition of the acceleration of the Mg^{2+} dependent HMM-S1 ATPase of F-actin by DNAase I*

Fig.3 illustrates the effect of increasing DNAase I concentration on the acceleration of the HMM-S1-ATPase by F-actin. About 40% inhibition is reached at a molar ratio of actin : DNAase I of about 4, whereas at equimolar ratio independent of the presence of tropomyosin there is full inhibition of actin activity. In a further set of experiments F-actin at low concentrations was incubated with increasing

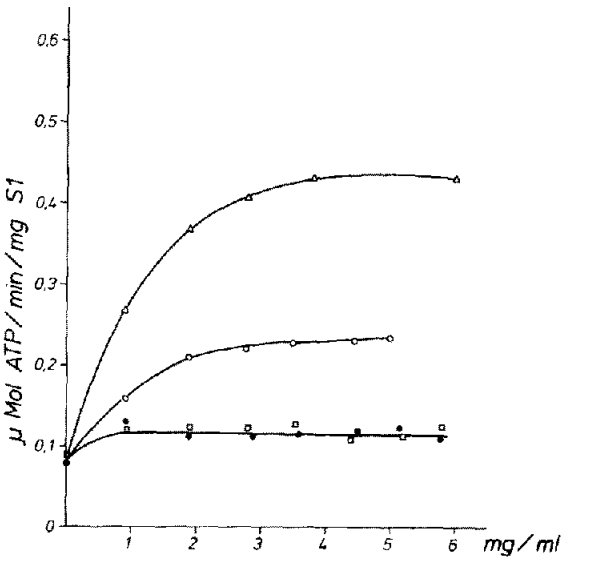


Fig.3. Inhibition of the acceleration of the HMM-S1 ATPase by DNAase I. (△——△) Pure F-actin no DNAase I added; (○——○) F-actin: DNAase I ratio 4; (□——□) pure F-actin plus equimolar DNAase I; (●——●) F-actin plus one seventh molar equivalent tropomyosin, actin: DNAase ratio 1. Abscissa: amount of actin added, ordinate: rate of ATP hydrolysis given in µmol ATP/min/mg HMM-S1.

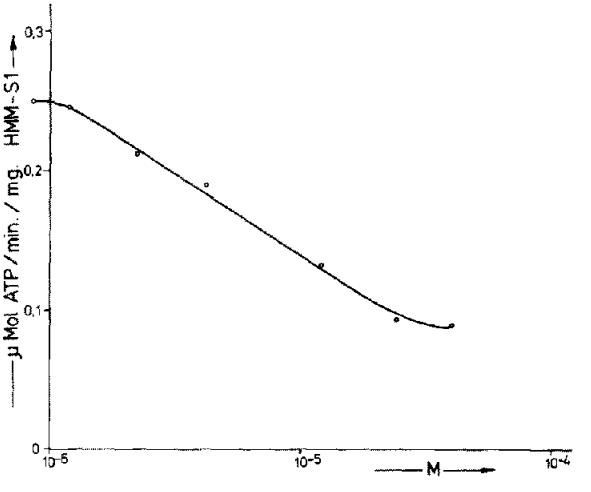
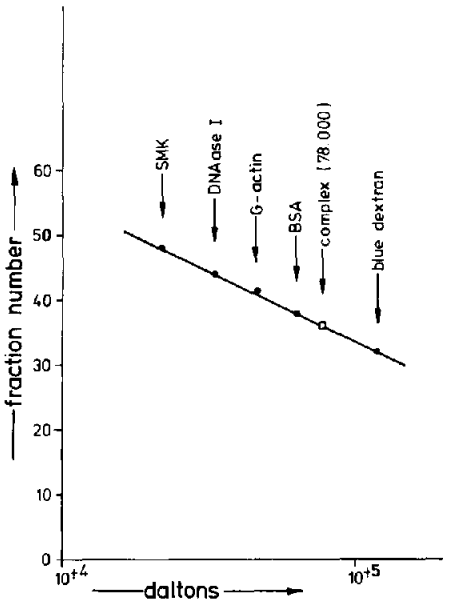


Fig.4. Semilogarithmic plot of HMM-S1 ATPase acceleration by F-actin with increasing amounts of DNAase I added. Ordinate: ATPase activity in µmol/min/mg HMM-S1; abscissa: final concentration of DNAase I added in essay containing $5 \cdot 10^{-6}$ M F-actin and 0.05 mg/ml HMM-S1. Reaction solution contained: 1 mM Mg ATP; 0.8 mM NADH; 2.5 mM PEP; 0.05 mg pyravate kinase; 0.05 mg lactate dehydrogenase in 1 ml. ATPase activity of actin-HMM-S1 without DNAase I: 0.25 µmol/min/mg HMM-S1.



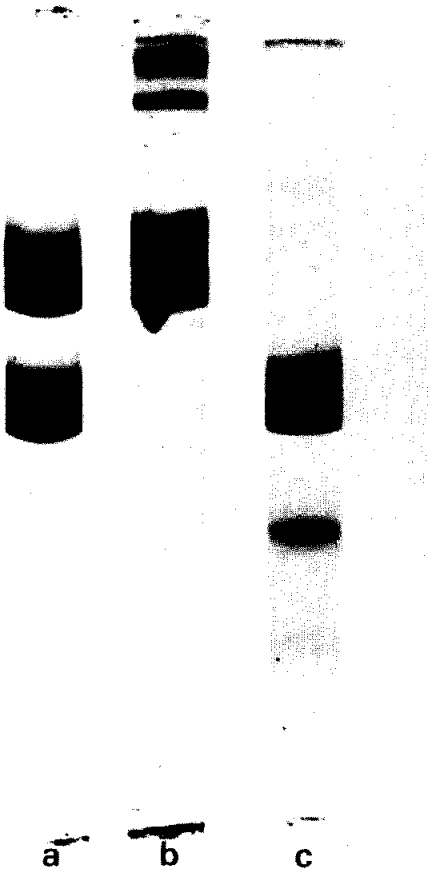


Fig.5. Molecular weight determination of DNAase I-actin complex on Sephadex G-100 calibrated with standard proteins indicated on the figure. Elution medium contained 30 mM KCl, 0.1 mM $CaCl_2$, 0.1 mM NaN_3, and HEPES buffer pH 7.5. Polyacrylamide gel in the presence of SDS: (a) protein peak; (b) actin; (c) DNAase I.

amounts of DNAase I for at least one hour and its effect on the Mg^{2+}-dependent HMM-S1-ATPase was tested. The results for $0.5 \cdot 10^{-5}$ M F-actin are shown in fig.5. At these concentrations 50% inhibition is reached at about equimolar concentration of DNAase I and actin, indicating a binding constant of about $1.8 \cdot 10^{5}$ M^{-1}.

3.3. *Determination of the molecular weight of the actin–DNAase I complex*

To examine the stoichiometry of the complex formation between DNAase I and actin more closely it was essential to determine the mol. wt of the complex. Therefore DNAase I was incubated with F-actin for one hour at equimolar or excess concentration and filtered through a calibrated Sephadex G-100 column. When mixed at equimolar concentration a single protein peak appeared, when DNAase I was present at excess two protein peaks appeared the first of which being identical in position to the single one. From the calibration (fig.5) its mol. wt can be estimated to be about 78 000. Polyacrylamide gels in the presence of SDS over the whole peak reveal two bands of mol. wt 43 000 and 33 000 identical to actin and DNAase I when run separately (fig.5). When DNAase I was in excess over actin the second peak gave a single band on gels corresponding to DNAase I.

4. Discussion

We have shown that DNAase I directly effects the polymerization of actin in the presence of ATP, forms a stoichiometric complex with G-actin subunits and competitively displaced HMM-S1 and tropomyosin

separately from the actin polymer. Conversely the DNAase I–actin complex can be dissociated by the joint presence of HMM-S1 and tropomyosin, indicating that the DNAase I reaction is fully reversible. In an independent study using ϵATP–G-actin nucleotide dissociation from G-actin by DNAase I interaction could be excluded, in fact the rate of nucleotide dissociation is reduced by a factor of 10 when the actin–DNAase I complex is formed (to be published). A biological role associated with the ability of DNAase I to depolymerize F-actin remains to be elucidated. In this connection it is interesting to note that in a number of eukaryotic cells, actin is present in the globular form at high concentration [9,10]. Since the mechanical function of actin is probably first realized after polymerization into the F-form it may be that this process is affected by DNAase I. Although DNAase I is regarded as an extracellular protein secreted by the pancreas, there exist a number of intracellular DNAases [11]. The ability to depolymerize F-actin may be used as a simple test to investigate other intracellular DNAases.

Note added in proof: Recently we learned that Drs S. E. Hitchcock and U. Lindberg independently obtained similar results.

References

[1] Lindberg, U. (1967) Biochemistry 6, 343–347.
[2] Lazarides, E. and Lindberg, U. (1974) Proc. Nat. Acad. Sci. USA, 71, 4742–4746.
[3] Szent-Györgyi, A. (1947) Academic Press, N.Y.
[4] Price, P. A., The-Yung, L., Stein, W. H. and Moore, S. (1969) J. Biol. Chem. 244, 917–923.
[5] Lowey, S., Slayter, H. S., Weeds, A. G. and Baker, M. (1969) J. Mol. Biol. 42, 1–29.
[6] Bailey, K. (1948) Biochem. J. 43, 271.
[7] Ziegler, A., Harrison, S. C. and Leberman, R. (1974) Virology 59, 509–515.
[8] Trentham, D. R., Bardsley, R. G., Eccleston, J. F. and Weeds, A. G. (1972) Biochem. J. 126, 635–644.
[9] Lazarides, E. and Weber, K. (1974) Proc. Nat. Acad. Sci. USA 71, 2268–2272.
[10] Lengsfeld, A. M., Löw, I., Wieland, T., Danker, P. and Hasselbach, W. (1974) Proc. Nat: Acad. Sci. USA 71, 2803–2807.
[11] Laskowski, M. (1967) Advances in Enzymology, Interscience Publishers N.Y. (F. F. Nord. ed.) 29, 165–220.