ACTIN FILAMENT DISASSEMBLY IN BLOOD PLASMA

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1. Introduction

Biochemical [1–3] and immunofluorescent [4,5] observations indicate that actin filaments are disrupted in plasma and serum. An actin depolymerizing protein has been partially characterized in human plasma and serum using the abolition of immunofluorescence as an assay [4,5]. A more quantitative assay may be achieved using the inhibition of pancreatic DNase I by actin monomers [6–8]. Here, this technique has been used to quantitate actin depolymerizing activity in plasma and serum and to characterize the stability of the factor involved. This factor is labile at 50°C or in the presence of papain indicating that it is a protein. The amount of actin depolymerized by plasma is ~100 μg/ml and the activity is unaffected by clotting.

2. Materials and methods

Rabbit or chick skeletal muscle actins were prepared from acetone powders [9]. Platelet actin was prepared from profilactin [10] by addition of EGTA to 5 mM [11] and sedimentation at $10^5 \times g$. Plasmas (platelet-rich and platelet-free) were obtained by differential centrifugation of pig blood [10] pooled from several animals. Rabbit plasma was obtained by an identical procedure. Platelet free plasma could be stored at −20°C for several weeks. Serum was prepared from plasma by addition of $CaCl_2$ to 1 mM followed by centrifugation to remove the clot. Fresh frozen human plasma was obtained from The Regional Blood Transfusion Centre, Cambridge.

The DNase inhibition assay for actin depolymerizing activity is described in [8]. F-Actin (30–100 μl at 200 μg/ml in 15% glycerol, 30 mM NaCl, 20 mM imidazole–HCl pH 7.0) is mixed with sample in a 1 ml, 1 cm path spectrophotometer cuvette; 0.5–1 min later 1–2 μg DNase are added, followed by 0.9 ml DNA ($A_{260}^{1\,cm} = 1.0$), at 25°C and in 125 mM Tris–HCl (pH 7.5), 5 mM $MgCl_2$, 2 mM $CaCl_2$. One unit of actin depolymerizing activity inhibits a DNase activity of 1 A_{260} unit . min^{-1} . cm^{-1} by 50%.

Radiolabelled actin was prepared either by incorporation of [α-^{32}P]ADP during polymerization or by reaction of F-actin with iodo-[$^{14}C_2$]acetamide [7]. Sedimentation of F-actin in the presence of plasma or suitable control buffers (30 mM NaCl, 20 mM imidazole–HCl (pH 7.0), 1 mM NaN_3) was done at $> 10^5 \times g$ in a Beckman airfuge or following addition of filamentous rabbit myosin in an MSE microfuge.

Aliquots of plasma and serum treated F-actin were applied to collodion and carbon-coated copper grids, stained with 1% uranyl acetate and observed with a Philips 400 electron microscope.

3. Results

3.1. *Evidence that filaments are depolymerized in plasma*

There are several lines of evidence that actin filaments are depolymerized in plasma. The best derives from the fact that actin monomers produced rapidly inhibit DNase activity but F-actin does not [6–8]. The experiment described in fig.1 shows that plasma alone does not inhibit DNase nor have DNase activity; however, when plasma is mixed with an excess of

Abbreviation: EGTA, ethylene glycol bis (β-aminoethyl ether) *N,N,N',N'*-tetraacetic acid

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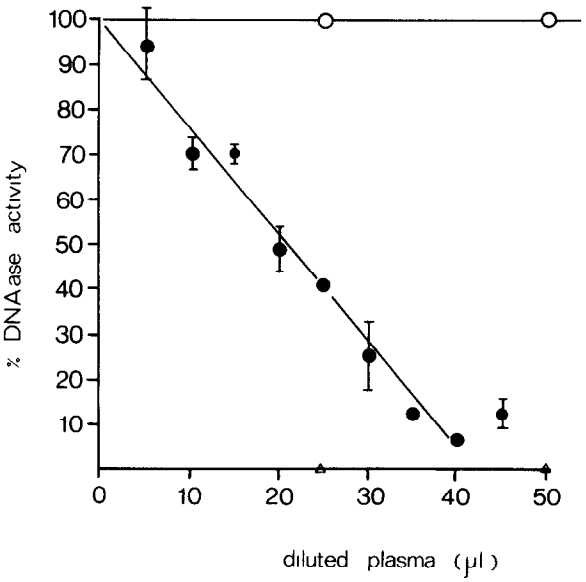

Fig.1. DNase inhibition by pig plasma is dependent on the presence of F-actin. (•) F-actin 5 μg in 100 μl, 30 mM NaCl, 20 mM imidazole–HCl (pH 7.0) mixed with increasing volumes of a 1:10 dilution of pig plasma; after 1 min, 1 μg DNase added. (0) Control without F-actin; 2 μg DNase mixed with a 1:5 dilution of pig plasma. (△) Control without actin or DNase; pig plasma diluted 1:5. In all cases, 0.9 ml DNA added.

F-actin, inhibition occurs in proportion to the amount of plasma added. This implies that F-actin is converted to monomers or into a form which inhibits DNase within the time required to set up the assay (<15 s). Supporting evidence comes from the observation that F-actin labelled either with [α-^{32}P]ADP or iodo-[$^{14}C_2$] acetamide is not sedimented at $> 10^5 \times g$ in the presence of plasma (94% of the radioactive phosphate remains in the supernatant compared to 12% in controls). Nor does the addition of filamentous myosin aid the sedimentation of radioactive actin in plasma treated samples. Similar experiments using ^{125}I-labelled F-actin have been reported in [5]. Finally, filaments can no longer be visualized by negative staining after F-actin has been mixed with a dialyzed, ammonium sulphate fraction (40–60% cut) containing the actin depolymerizing activity. Serum is reported to have a similar effect [5].

The actin depolymerizing activity is not specific for the polymorphic form of actin. Rabbit and chick skeletal muscle actins which are both α-forms [12] and platelet actin which contains both β- and γ-forms [10] are depolymerized to a similar extent.

3.2. *Quantitation of the actin depolymerizing capacity of plasma and serum*

The specific activity of pig plasma is typically ~ 1 unit/mg protein and 60 units/ml (table I). This corresponds to ~ 100 μg actin depolymerized/ml plasma. Clotting of platelet-rich or platelet-free pig plasma had no significant effect on the level of actin-depolymerizing activity remaining in the serum. Somewhat lower activities were measured for human and rabbit plasma.

3.3. *Characterization of the depolymerizing factor*

The actin depolymerizing factor was partially purified following exhaustive dialysis in 0.14 M NaCl, 20 mM imidazole–HCl (pH 7.0) (100% activity recovered), by ammonium sulphate fractionation (30–40% cut) and gel filtration on Sephadex G-100 in the same buffer. The activity eluted with M_r = 100 000 and a 5-fold enhancement of specific activity was achieved. Actin depolymerizing activity was destroyed when this material was incubated with papain (1/150, w/w, at 25°C) with a $t_{1/2}$ of 16 min.

The depolymerizing activity is heat-labile. In agreement with [5] activity was completely destroyed

Table 1
Quantitation of actin depolymerizing activity in plasma and serum

Sample	No. used	Specific activity (units/mg)	Specific activity (units/ml)	Actin depolymerized (μg/ml)
Pig plasma	1	1.51	81.3	132
Pig plasma	7	1.16±0.24	63.6±14.3	101[a]
Rabbit plasma	1		22.0	35.7[a]
Human plasma	5	0.72±0.09	38.5± 2.2	62.6[a]
Platelet-rich plasma-derived serum (pig)	1		80.6± 4.0	131[a]
Platelet-free plasma-derived serum (pig)	1		83.3± 4.2	135[a]
Control plasma for serum samples (pig)	1		75.3± 3.8	122[a]

[a] Calculated from the activity of the first plasma sample which was calibrated with a standard G-actin solution

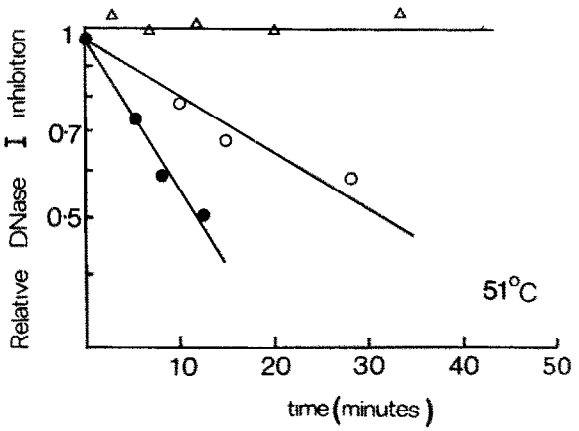

Fig.2. Heat inactivation of plasma actin depolymerizing activity. Partially purified depolymerization factor (see text) was incubated at 51°C in 0.14 M NaCl, 20 mM imidazole–HCl (pH 7.0) without addition (•), with 20% glycerol (○), or with 1 mM $CaCl_2$ (△). At intervals, aliquots (50 μl) were assayed for DNase inhibition using 100 μl F-actin (0.23 mg/ml) and 2 μg DNase.

within 30 min at 56°C. Using the partially purified material described above, no decay in activity was observed at 40°C over 40 min. Fig.2 shows experiments at 51°C where the $t_{1/2}$ of 12 min is increased to 31 min in the presence of 20% glycerol and 1 mM $CaCl_2$ provides complete stabilization over 40 min. Further experiments showed that the $t_{1/2}$ was ~ 2.5 h in 10^{-4} M $CaCl_2$ but 10^{-5} M $CaCl_2$ provided no stabilization.

4. Discussion

Inhibition of pancreatic DNase by depolymerized actin provides a quantitative means of measuring actin depolymerizing activity. The assay may be used with muscle or non-muscle F-actin and functions in the presence of complex biological fluids such as plasma. Partial purification of the depolymerizing activity in plasma indicates that the active component is a protein of 100 000 M_r which is labile to heating at 51°C but stabilized under these conditions in the presence of 10^{-4} M $CaCl_2$. An intracellular actin-depolymerizing protein (M_r <35 000) isolated from brain has been reported in [13]. In contrast to the plasma protein, Ca^{2+} increase the heat-lability of this brain factor. The DNase assay shows that pig plasma contains sufficient factor to depolymerize 100 μg F-actin/ml. Similar quantitation [13] of the amount of factor in chick embryo brain indicates that up to 400 μg F-actin can be depolymerized/g brain tissue.

Whilst the function of the brain depolymerizing protein may be to aid in the recycling of intracellular F-actin, the reason for the presence of such a factor in plasma remains unclear. It may serve to disassemble filamentous actin released by platelets or other blood cells since the highly viscous nature of F-actin would be deleterious to blood flow through the capillaries. Alternatively the protein may be transported in the blood and taken up into cells.

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