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Instability of F-actin under the influence of β-actinin

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SUMMARY

When G-actin was polymerized in the presence of β-actinin, the resultant F-actin became unstable on incubation in 0.1 M KCl at 25 °C. The F-actin filaments changed into globular aggregates of 20–30 nm in diameter. ATP protected the denaturation of β-actinin-modified F-actin. Thus the instability of the isolated I filaments is explained by the influence of β-actinin.

Natural F-actin, prepared from rabbit skeletal muscle without the depolymerization step[1], has been reported to be unstable in 0.1 M KCl, and ATP protects its denaturation[2,3]. The F-actin separated from natural actomyosin (myosin B) is also shown to be easily denaturated on incubation at 25 °C in 0.1 M KCl[4]. On the other hand, usual F-actin preparations of the Straub type are remarkably stable in 0.1 M KCl[2,3]. Is this property of natural F-actin characteristic of actin which is not subjected to acetone treatment? The present study has revealed that β-actinin, a minor regulatory protein of muscle[5–7], is responsible for the instability of F-actin. It has been suggested that β-actinin is bound to F-actin as an ending factor[8], and the subsequent study has shown that β-actinin is bound to one end of F-actin resulting in changes in some properties of F-actin[9].

G-Actin was purified by a slight modification[10] of the Mommaerts procedure[11]. β-Actinin was highly purified from rabbit skeletal muscle by a DEAE-Sephadex chromatography[12]. Flow birefringence was measured at a velocity gradient of 1000 s^{-1} in an apparatus of the Edsall type (Rao Instrument Co.). The removal of free ATP from a G-actin solution was effected by a use of Dowex-1 resin (Cl^- form)[13].

As shown in Fig. 1, F-actin polymerized in the presence of β-actinin (5% by wt to actin) showed a marked decrease in the degree of birefringence on incubation at 25 °C, whereas F-actin alone, or F-actin to which β-actinin was added after polymerization, hardly showed any decrease up to 3 h of incubation. In the presence of ATP, the decrease,

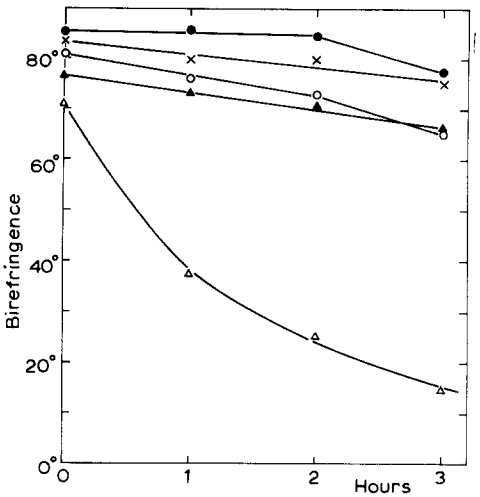

Fig. 1. Stability of β-actinin-modified F-actin. F-actin, 0.25 mg/ml, was incubated at 25 °C in the presence of 0.1 M KCl and 10 mM Tris buffer, pH 7.2. Dowex-1-treated G-actin (1 mg/ml) was polymerized by 0.1 M KCl without (control) or with β-actinin 0.05 mg/ml (β-actinin modified). ATP was added to F-actin to a final concentration of 0.5 mM, when indicated. Birefringence was measured at a velocity gradient of 1000 s^{-1}. ○, control; ●, control + ATP; △, β-actinin-modified F-actin; ▲, β-actinin-modified F-actin + ATP; ×, control (F-actin) + β-actinin, 5% by wt of F-actin.

if any, in birefringence occurred to a small extent. In this experiment, any free ATP had been eliminated from G-actin by the Dowex-1 treatment. The decrease in birefringence was accompanied by the appearance of globular aggregates of actin, 20–30 nm in diameter, as revealed by electron microscopic examinations (Fig. 2). The remaining fibrous form of actin had largely deteriorated in the fine structure, although double-stranded helical structure was locally retained. Aggregate, once formed, was not repolymerized to F-actin after the depolymerization procedure.

It was observed that β-actinin was fully effective in giving F-actin instability, even at such small amounts as 1% of actin by wt (Fig. 3). Amounts as great as 10% of β-actinin did not show any further effects on the instability of β-actinin-modified F-actin. 0.1 mM ATP completely protected the β-actinin-modified F-actin from the denaturation, and a half-maximal protection was achieved by $2 \cdot 10^{-5}$ M ATP (Fig. 4). These situations were almost the same as in the case of natural F-actin[3]. However, a difference was observed between natural F-actin and β-actinin-modified F-actin: for the former the instability was independent of protein concentration up to 0.75 mg/ml (ref. 2). On the other hand, β-actinin-modified F-actin became more stable as the protein concentration was increased, as shown in Fig. 5.

β-Actinin is sensitive to tryptic digestion[5,7], whereas actin is resistant[1,7]. Therefore, β-actinin-modified F-actin was treated by trypsin to remove β-actinin. There was not much change in the instability by the trypsin treatment, as shown in Table I. However, when the trypsin-treated sample was subjected to sonic vibration, the resultant F-actin

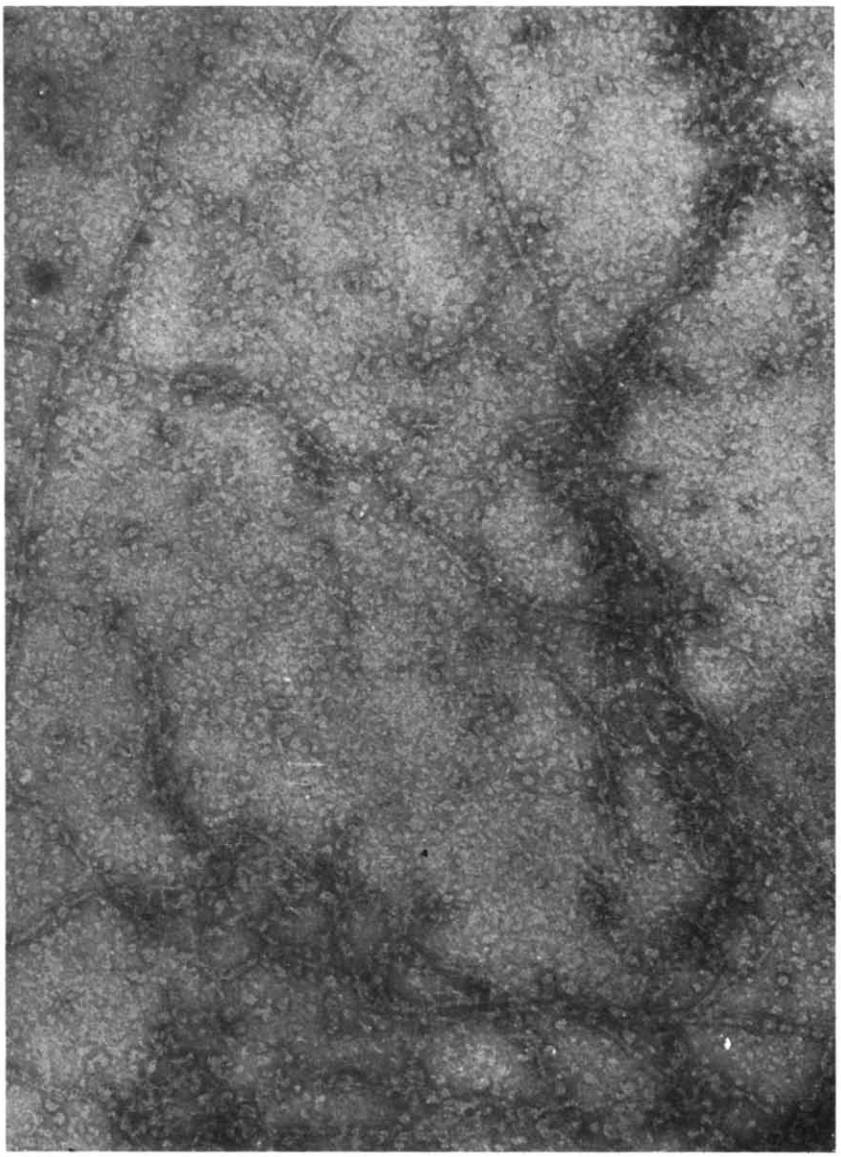

Fig. 2. An electron micrograph of β-actinin-modified F-actin incubated for 3 h in 0.1 M KCl. Conditions, as in Fig. 1. Negatively stained with uranyl acetate. Magnification, × 90 000.

became as stable as control F-actin (Table I). These observations suggest that some structural changes of F-actin, induced by polymerization in the presence of β-actinin, persist after the removal of β-actinin, but they are converted to the Straub-type state of F-actin on further treatment with sonic vibration. This interpretation was supported by the fact that an addition of β-actinin to preformed F-actin did not cause any instability of the F-actin

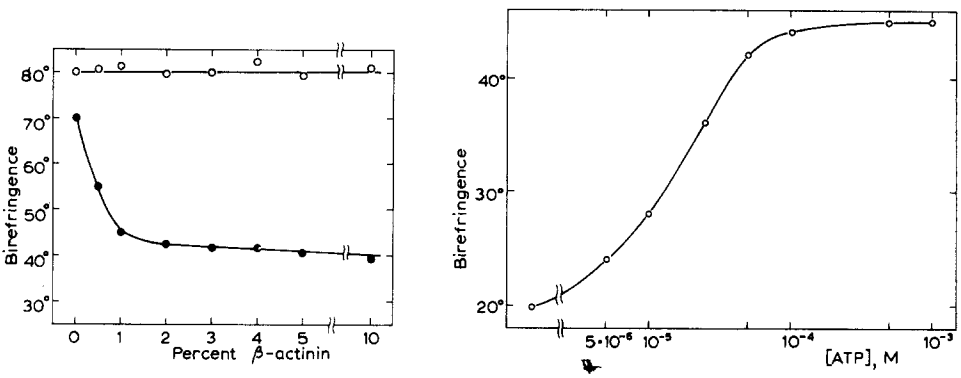

Fig. 3. Effect of various amounts of β-actinin on the stability of F-actin. The conditions were the same as in Fig. 1, except that G-actin was polymerized in the presence of various amounts of β-actinin, as indicated on the abscissa. Measurements were performed before (○) and after 2 h of incubation (●). ATP was not added.

Fig. 4. Effect of ATP concentration on the stability of β-actinin-modified F-actin. Conditions were the same as in Fig. 1 except that F-actin polymerized with 5% β-actinin, 0.15 mg/ml, was incubated for 2 h in the presence of various concentrations of ATP, as indicated on the abscissa.

TABLE I

EFFECT OF β-ACTININ ON THE STABILITY OF F-ACTIN

F-actin, 0.20 mg/ml, was polymerized with or without 5% β-actinin. Trypsin treatment was performed for 15 min at 25 °C (trypsin, 1% of actin by wt). Sonication was carried out for 10 s at 15 °C in a Tomy sonicator (20 kcycles). When added, the concentration of ATP was 0.5 mM. Other conditions, as in Fig. 1.

System	*ATP*	*Degree of birefringence*	
		Start	*After 2 h at 25 °C*
F-Actin	–	58°	55°
	+	58°	56°
F-Actin polymerized	–	55°	26°
with β-actinin	+	57°	54°
treated with trypsin	–	50°	30°
	+	55°	52°
treated with trypsin,	–	55°	54°
then sonicated	+	55°	51°
F-actin, β-actinin	–	57°	54°
added after polymerization	+	58°	57°

(Table I, see Fig. 1). It is to be noted that Mg polymer, a special form of F-actin, is formed when G-actin is polymerized by 1–2 mM $MgCl_2$ in the presence of β-actinin[14].

Whatever the mechanism of the instability of F-actin caused by β-actinin may be, it is of special interest that the I filaments are very likely formed *in vivo* under the influence

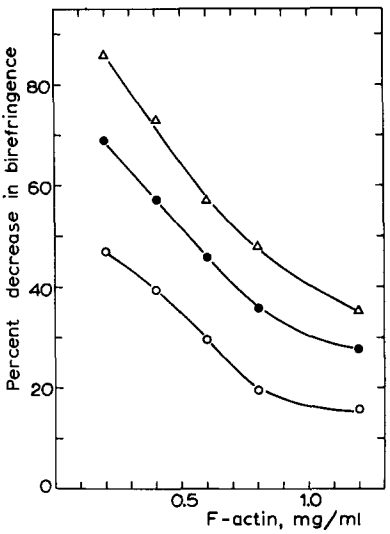

Fig. 5. Effect of protein concentration on the stability of β-actinin-modified F-actin. Conditions were the same as in Fig. 1 except for varied concentration of F-actin polymerized with 5% β-actinin. ○, incubated for 2.5 h; ●, 5 h; △, 7.5 h.

of β-actinin. The possible role of β-actinin during myogenesis was already pointed out in a previous paper [15].

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