Polymerization of β-like actin from scallop adductor muscle

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Scallop adductor muscle β-like isoactin differs from rabbit skeletal muscle α-actin in the rate, extent and critical concentration of polymerization. The difference is temperature- and [KCl]-dependent. In the presence of DNase I scallop actin was shown to be depolymerized more rapidly than rabbit actin. It was suggested that the polymers formed by β-actin are less stable than those formed by α-actin.

(Scallop) Actin Isoactin Polymerization Depolymerization DNase I

1. INTRODUCTION

It is well known that actin is represented in different muscles and non-muscle cells by a variety of isoforms. Skeletal muscles of vertebrates contain mostly α-actin, while β- and γ-actins are the main isoforms of smooth muscles and cytoskeleton [1,2]. Several amino acid replacements are found in the primary structure of these isoactins, some of which are located at the amino-terminal end of the molecule [3]. It has been suggested that the N-terminal part of the actin molecule must be involved in actin polymerization, since chemical modification in this region of skeletal muscle actin affects this property [4–6] and muscle actin devoid of its amino-terminal amino acid residues does not polymerize [7,8]. It is reasonable therefore to expect that isoactins can be distinguished by their polymerizability.

Differences in polymerization properties of actins from different muscles have been demonstrated [9,10], however all these actins consisted of several species. Only indirect data are available concerning the polymerization of individual isoforms of actin [11,12]. Here, we used actin isolated from scallop adductor muscle as a model of β-actin. Scallop actin was shown previously to be the β-like isoactin and the only actin species present in scallop adductor muscle [13].

We demonstrate here that the rate and extent of scallop adductor muscle β-like actin polymerization are lower while the rate of its depolymerization is higher than that of rabbit skeletal muscle α-actin. The critical concentration for scallop actin polymerization is higher than that of rabbit actin when actins are polymerized with low KCl concentrations or at low temperature.

2. MATERIALS AND METHODS

Scallop actin was isolated from acetone-dried myofibrils prepared from red (striated) adductor muscle of the scallop *Patinopecten jessoensis* as described [14] and washed with 5 mM $NaHCO_3$ before acetone treatment. Skeletal muscle actin was extracted from acetone-dried residue of myofibrils after treatment with high ionic strength solution [15]. Both proteins were purified by one cycle of polymerization-depolymerization using 30 mM KCl for polymerization at room temperature overnight [9]. F-Actin is known to be stable to trypsin digestion whereas G-actin and other proteins are trypsin-sensitive [7,16]. Therefore, to remove actin-binding proteins, both actins in the polymeric form were treated with trypsin. Trypsin (Sigma) was added to F-actin at a ratio of 1:200 (w/w). Digestion proceeded for 1 h

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at room temperature and was terminated by addition of soybean trypsin inhibitor followed by sedimentation of actin at 100000 × *g* for 2 h. Pellets were depolymerized by homogenization and subsequent dialysis against extraction solution (0.5 mM ATP, 0.2 mM $CaCl_2$, 0.01% NaN_3, 2 mM Tris-HCl; pH 7.5) after which actins were clarified at 150000 × *g* for 1 h. The final G-actin preparations were electrophoretically homogeneous and according to the fluorescence data [17] did not contain any inactivated actin.

Viscosity was measured in the Ubellode type viscometer with an outflow time for water of 93 S at 20°C.

3. RESULTS

3.1. *Kinetics of actin polymerization*

The time course of polymerization was determined by viscosity measurement. As shown in fig.1, the rate and extent of polymerization of actins from both scallop adductor and rabbit skeletal muscle depended on the experimental conditions. In 0.1 M KCl, 1 mM $MgCl_2$ (salt concentrations which are optimal for actin polymerization) the rates of polymerization of both actins were identical. However, the final viscosity of the solution of scallop actin was lower than that of rabbit actin (fig.1a). This difference increased when KCl in the solution was far from the optimal concentration. For example, in 0.02 M KCl the rate of polymerization of scallop actin and final viscosity of the solution were 1.5–2-times lower than those of rabbit skeletal muscle (fig.1b). The same effect was observed when the actins were polymerized in 0.1 M KCl without $MgCl_2$ (fig.1a).

3.2. *Depolymerization of F-actins with DNase I*

DNase I has been shown to depolymerize F-actin in solution [18]. Although the exact mechanism of this reaction is unclear the rate of depolymerization could be a measure of the stability of actin polymers. Therefore, we were interested in comparing depolymerization of scallop and rabbit actins by DNase I. It turned out that even in 0.1 M KCl the rate of depolymerization of scallop F-actin with DNase I was higher than that of rabbit F-actin (fig.2). The difference was not very large but was reproducible in every series of experiments. We suggested that this difference should increase in

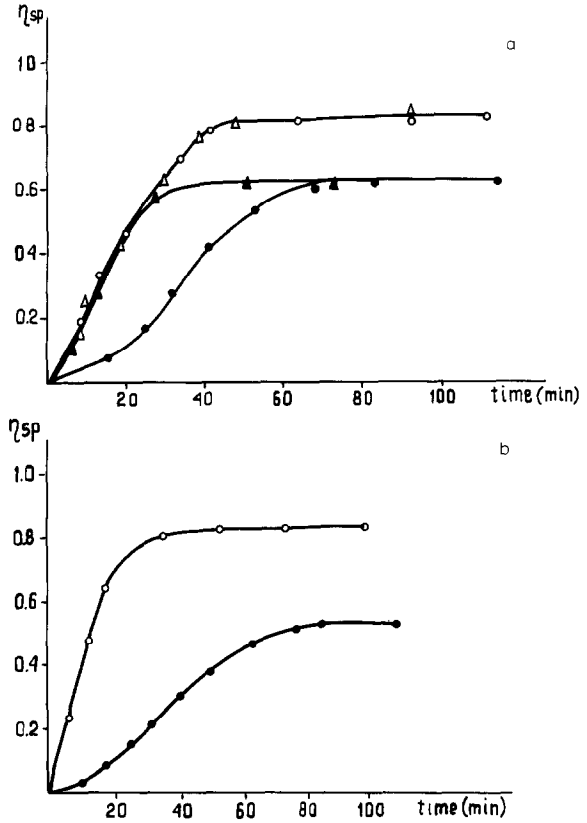

Fig.1. Time course of polymerization of scallop and rabbit actins assayed by viscometry. Conditions: 0.5 mg/ml of actin, 0.5 mM ATP, 0.2 mM $CaCl_2$, 0.01% NaN_3, 2 mM Tris-HCl, pH 7.5. Conditions for polymerization: (a) 0.1 M KCl in the presence (△, ▲) and absence (○, ●) of 1 mM $MgCl_2$; (b) 0.02 M KCl; 20°C. (●, ▲) Scallop actin, (○, △) rabbit actin.

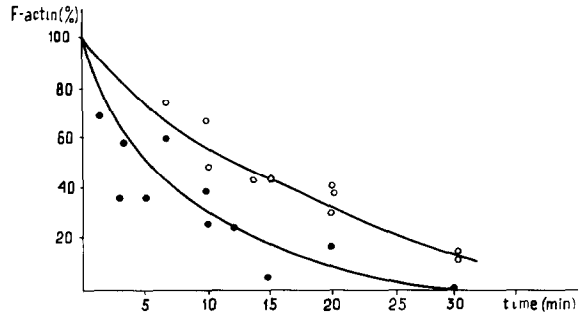

Fig.2. Depolymerization of scallop and rabbit F-actins with DNase I. F-Actins (1 mg/ml) in 0.1 M KCl were mixed with DNase I (1 mg/ml) at a ratio of 1:1 (w/w). In the indicated time intervals, aliquots of the mixture were added to the DNA solution to measure the amount of G-actin according to [19]. Each curve represents the average of 3 different experiments. Conditions and symbols as in fig.1.

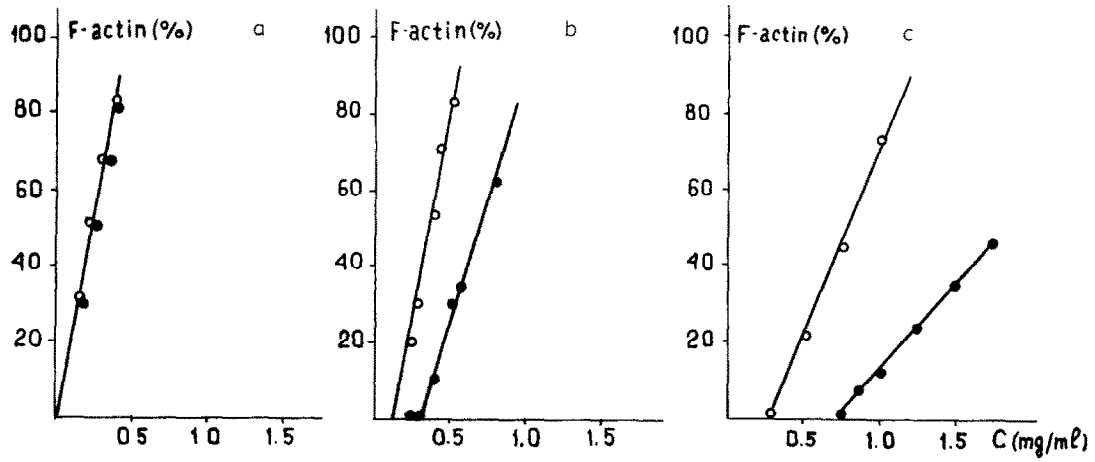

Fig.3. Quantity of scallop and rabbit F-actins as a function of actin concentration assayed by the sedimentation procedure. Conditions for polymerization: (a) 0.1 M KCl, 20°C; (b) 0.1 M KCl, 4°C; (c) 0.02 M KCl, 4°C. Other conditions and symbols as in fig.1. In the sedimentation experiments samples were centrifuged at 100000 × *g* for 2 h and the quantity of F-actin calculated as the difference in protein concentration of initial sample and supernatant.

0.02 M KCl as was the case for actin polymerization. However, we failed to make such a comparison because both F-actins were depolymerized with DNase I in 0.02 M KCl too rapidly.

3.3. *Critical concentration for polymerization*

The amount of F-actin in preparations was determined by sedimentation. Samples of scallop and rabbit actin were polymerized with 0.02 and 0.1 M KCl at 4 and 20°C overnight. The critical concentration for polymerization was determined from the abscissa intercept of plots of the quantity of F-actin vs actin concentration (fig.3). Whereas the critical concentration for polymerization of both actins polymerized with 0.1 M KCl was the same at 20°C (fig.3a), at 4°C it was higher for scallop than for rabbit actin (fig.3b). The difference in critical concentration became larger when the actins were polymerized with 0.02 M KCl (fig.3c).

4. DISCUSSION

The viscosimetric and sedimentation studies performed in here have shown that the critical concentration for scallop adductor muscle β-like actin polymerization is higher than that of rabbit skeletal muscle α-actin. Moreover, the rate and extent of scallop β-actin polymerization were lower compared with those of rabbit α-actin. Since we used highly purified actins these results seem to be due not to the presence of any actin-binding proteins but to the properties of actins themselves. It is important from this point of view that scallop and rabbit actins differed very little under physiological conditions where the influence of actin-binding proteins might be strongest.

The difference became greater on lowering of the ionic strength or temperature of the solution. Similar results have been obtained for gizzard actin [9] and actin from muscle layer of bovine aorta [10].

The difference in critical concentration and extent of polymerization suggests that polymers formed by rabbit skeletal muscle α-actin are more stable than those from scallop adductor muscle β-like actin. This suggestion is supported by the fact that in the presence of DNase I scallop actin depolymerized more rapidly than skeletal muscle actin.

It is well known that the cytoskeleton of non-muscle cells is a dynamic system of microfilaments which can assemble or disassemble during different cell processes [20–22]. In contrast, skeletal muscle myofibrils are rather stable. The data obtained in this and other works [9–12] allow one to suppose that this stability or instability may be due not only to the presence of actin-binding proteins as has been widely discussed [23,24] but also to the different behaviour of α-, β- and γ-isoactins.

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