Preparation and Characterization of Actin from Liver

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G-actin was prepared from pork liver by gelfiltration on AcA 44 Ultrogel. The purity of the actin was 95−98% and the yield was 45−150 mg from 500 g liver, corresponding to a maximum efficiency of recovery of about 30%.

High viscosities of actin-enriched solutions were reduced by a specific pH-step. In SDS-gel electrophoresis a single band of about ~ 45000 daltons was seen. The G-actin was polymerizable as shown by high shear viscosity measurements. The isoelectric points of the β- and γ-isoactins were 5.6 and 5.7.

Introduction

Actins have previously been isolated from a variety of non-muscle sources. However, no procedure has yet been published dealing with the easily available liver of larger mammals, *e.g.* pigs.

Availability of liver actin was desirable for a number of reasons *e.g.* as antigen in, among others, immunohistochemical studies, *in vitro* experiments with toxins specific to liver, *e.g.* phalloidin [1], cytochalasin.

Antibodies against this non muscle-actin may help to elucidate the change of the cell surface when there is a malignant transformation [2, 3].

Materials and Methods

Biochemical were from Merck AG, Darmstadt and were of the highest purity available. Protein concentrations were estimated by the micromethod of Lowry *et al.* [4]. NaDoSO$_4$/polyacrylamide gel electrophoresis was used [5]. Reagents for isoelectric focusing were those specified by O'Farrell [6]. The pH gradient was established by 2% (w/v) ampholines of which 80% were pH 5−7 and 20% were pH 3.5−10. Focusing was performed at 200 V for 15 h followed by 400 V for 1 h.

Viscosity measurements were performed at high shear rates by using 2 ml capacity Oswald viscosimeters having outflow times for buffer G, 2 mM

Tris/HCl, 0.5 mM ATP, 0.08 mM CaCl$_2 \times 2$ H$_2$O, 0.1 mM DTE, of about 70 s at 298 K.

Actin filaments were visualized in the electron microscope by staining on rhodium plated, 400 mesh copper grids, covered with a carbon coated Formvar film. The grids were rendered hydrophobic by glow discharge treatment just prior to use. Briefly, 10 µl actin solution was adsorbed onto a grid for 1 min, excess solution removed and 2% phosphotungstic acid (pH 7.0) applied for 1 min. After removal of excess stain, grids were air dried and examined and photographed in a Zeiss-EM-10 CR electron microscope at 80 kV.

Results and Discussion

a) Preparation and purification of actin from pork liver

All procedures were carried out at 277 K.

I: Mincing and washing of pork liver at pH 7.0

Portions of 500 to 600 g fresh liver from pigs of approximately six months of age were minced and suspended in about 800 ml of buffer A: 15 mM HPO$_4^{2-}$/H$_2$PO$_4^-$, 5 mM NaCl, 3 mM DTE, 0.5 mM PMSF, 1 mM EDTA (pH 7.0). After homogenization (1)* for 90 s in an Ultra Turrax (10 000 rpm, Janke und Kunkel, FRG), the red suspension was washed four times with the same buffer, and centrifuged at 13 200×g for 30 min. The supernatant was decanted.

* Bracketed numbers refer to Table I.

II: Extraction of actin and actin-associated proteins at pH 8.7

The pellet was extracted with about 1 l buffer B: 0.5 M KCl, 5 mM KHCO$_3$, 2 mM DTE, 0.5 mM PMSF (pH 8.7) with vigorous stirring for 45 min. Two centrifugation steps followed: First, at 13 200 × g, second 30 min at 30 900 × g. The supernatants were pooled (2), filled into visking dialysis tubing and attached to a stirrer.

III: Pelleting of actin and actin-associated proteins at pH 6.5

A dialysis with gentle stirring against 10 l of buffer C: 15 mM Tris/Maleat, 1 mM DTE (pH 6.5) for 3 h followed. After the first hour, the stirring was briefly interrupted every 30 min to make sure that actin and actin-associated proteins precipitated quantitatively.

IV: Solubilizing of actin and actin-associated proteins at pH 7.6

After centrifugation for 30 min at 30 900 × g the light brown precipitate (20–40 ml) was suspended in 108 ml buffer D: 20 mM Tris/HCl, 0.6 M KCl, 0.5 mM ATP, 1 mM DTE, 0.5 mM PMSF (pH 7.6), homogenized for 30 s with a motor driven Teflon/glas homogenizer. KCl was added to a final concentration of 0.5 M. After ultracentrifugation at 100 000 × g for 1 h actin and actin-associated proteins were in the supernatant (3). The remaining pellet was treated as described above, however, with only 72 ml of buffer D (4).

V: Repetition of III

The supernatants were dialyzed against 2.4 l low ionic strength buffer C (pH 6.5).

VI: Dissociation of actin from actin-associated proteins at pH 7.5 and pH 4.2

The whitish pellet from this second dialysis step at pH 6.5 was suspended in buffer D$_1$: 20 mM Tris/HCl, 0.6 M KCl, 1 mM DTE, 0.5 mM PMSF, 5 mM ATP, 5 mM CaCl$_2$ × 2 H$_2$O (pH 7.5) by gentle homogenization. The suspension was clarified by centrifugation at 30 900 × g for 20 min (5), the protein concentration adjusted to approximately 5 g/l, and the solution, about 50 ml, dialyzed for about 3 h in buffer D$_1$ at pH 4.2 (Trial experiments at pH values 4.0 to 4.5 indicated pH 4.2 to be the most suitable), (Fig. 1). The pellet was discarded. The main component of the supernatant was actin (6).

VII: Concentration of the actin at pH 7.5

The pH of the supernatant was adjusted to pH 7.5. After dialyzing the actin-enriched solution for 6 h against buffer F: 5 mM Tris/HCl, 0.2 mM DTE, 2 mM NaN$_3$, 100 mM KCl, 0.5 mM ATP, 12 mM MgCl$_2$ × 6 H$_2$O, 0.2 mM CaCl$_2$ × 2 H$_2$O, 15 (w/v) PEG 6000 at pH 7.5, mainly actin was pelleted.
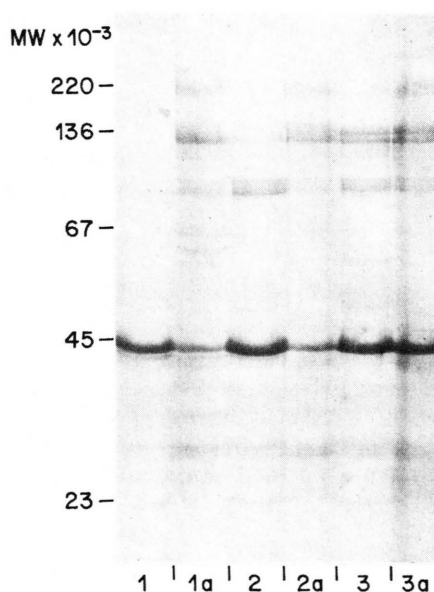

Fig. 1. Coomassie blue stained NaDodSO$_4$/8% polyacrylamide gel of G-actin preparation after dialysis in buffer D$_1$ for ~3 h at pH: 4.0 (1, 1 a), 4.2 (2, 2 a), 4.5 (3, 3 a). 1, 2, 3 supernatants, 1 a, 2 a, 3 a pellets.

Table I. Typical scheme of purification of pork liver actin.

Purification step	Volume [l]	Protein [g/l]	Total protein [g]
(1) Homogenate	1.2	27.1	32.52
(2) KCl-Extract	1.2	7.1	8.52
(3) 100 000 × g	0.08	6.5	0.52
(4) 100 000 × g	0.06	4.0	0.24
(5) before pH step	0.05	4.6	0.23
(6) after pH step	0.045	3.8	0.171
(7) after PEG step	0.013	10.2	0.133

VIII: Depolymerization and purification of pork liver actin at pH 7.5.

For depolymerization the pellet was solubilized in 10 to 15 ml buffer H: 20 mM Tris/HCl, 0.6 M KI, 5 mM ATP, 5 mM CaCl$_2$×2H$_2$O, 1 mM DTE (pH 7.5) and left for 1 h before centrifugation [7]. The resulting supernatant (7) was for an AcA 44 Ultrogel column, equilibrated with 350 ml buffer D. 20–30 ml buffer H were applied before and after loading it with a sample. The proteins were eluted with buffer D, elution time about 25 h.

IX: Increase of the actin yield

After the PEG-Step (see Table I) only 30% of the total protein is soluble in buffer H. Therefore the remaining pellet was resuspended in the same volume of buffer H. After centrifugation the supernatant was applied to another AcA 44 Ultrogel column. In this way the actin yield was increased by 2–3 fold. From 500 to 600 grams pork liver 45–150 mg polymerizable actin with a purity of 95% or higher were obtained. Assuming that the actin content in liver cells accounts for about 1.5% of the total cellular protein [8], the procedure described above allows a recovery of about 30% of pure actin.

In SDS-gel electrophoresis a single band of approximately ~ 45000 daltons was seen. Isoelectric focusing showed two bands with isoelectric points of 5.6 and 5.7 (Fig. 2). The relationship
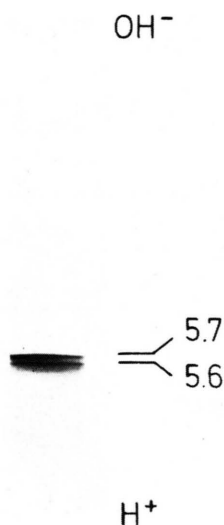
OH$^-$

5.7
5.6

H$^+$

Fig. 2. Isoelectric focusing of pork liver isoactins. IP β-actin: 5.6 IP γ-actin: 5.7.
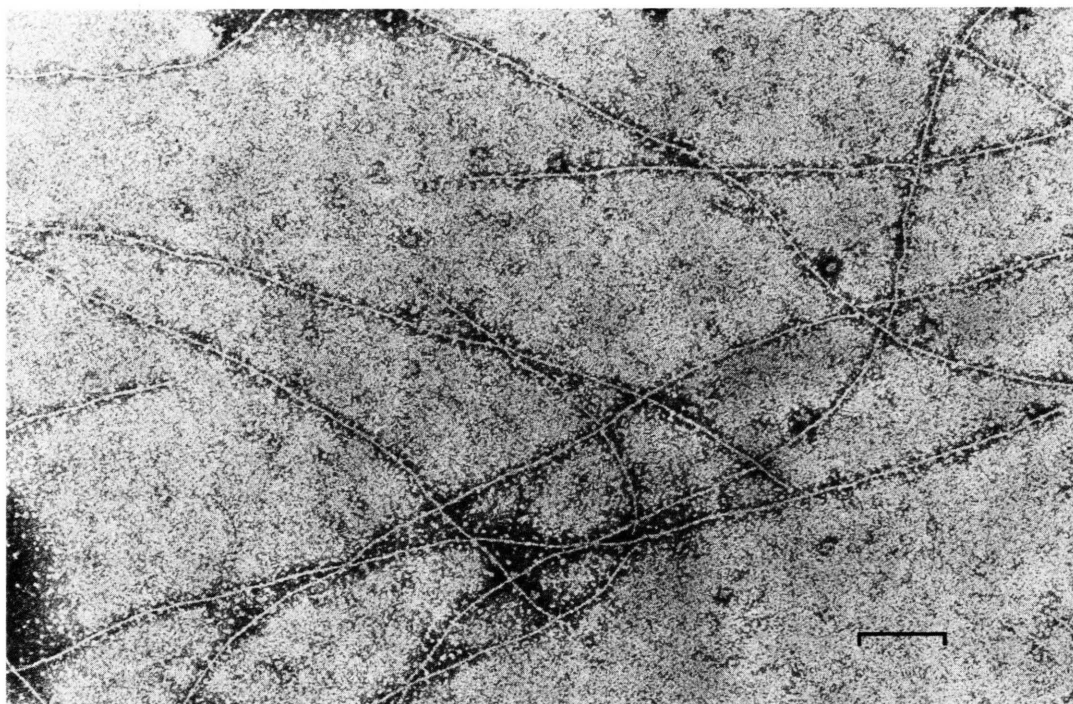

Fig. 3. Electron micrograph of liver actin microfilaments as visualized by negative staining. Actin (0.25 g/l) prepared as described, incubated with 100 mM KCl in buffer G for 10 min at 310 K. Final magnification 60 000 ×. Bar″‴= 200 nm.

between the β and γ-isoactins was 1:1, estimated from several isoelectric focusing experiments.

The actin was polymerizable as shown by Fig. 3.

Polymerization of pork liver actin in 2 mM Mg^{2+} and 100 mM KCl was not significantly different when compared to other non-muscle actins (Fig. 4).

The critical concentrations in 2 mM Mg^{2+} at 298 K and 278 K were about 0.03 g/l. However, in 100 mM KCl the critical concentrations increased from 0.06 g/l at 298 K to 0.26 g/l at 278 K.

As to the critical concentrations, the actin described here is in the range typical for other non-muscle actins [8], although the increase of the critical concentration of 278 K compared to the value of 298 K is only 4-fold.

From three critical concentrations the ΔH value for the G−F equilibrium in 100 mM KCl was calculated to be 50.2 ± 2.4 kJ/mol (Fig. 5).

The activation-enthalpy, determined by an Arrhenius-plot of half-polymerization time ($\tau_{1/2}$) versus the reciprocal of the absolute temperature ($1/T$), was 77.4 ± 2.0 kJ/mol.

The ΔH value is within the range reported for other non-muscle actins. The value for the activation enthalpy is not very different for the one reported for muscle actin [9].

Solutions of F-actin are non Newtonian fluids. At high shear rates, typically above 100 s⁻¹, the structure of the fluid is largely destroyed [10], as in-
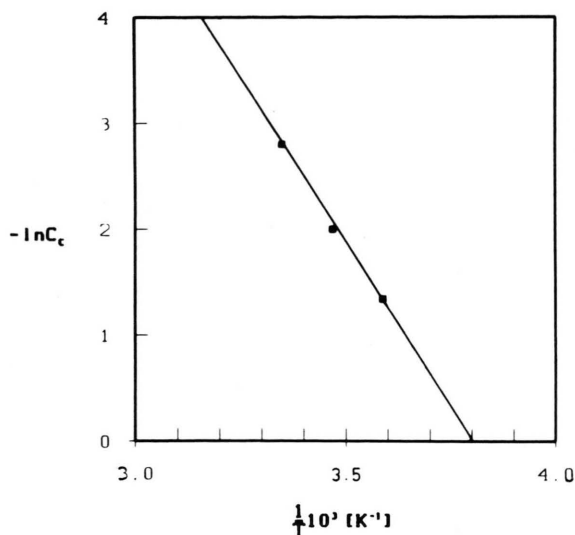

Fig. 5. The logarithm of the critical actin concentration as a function of the reciprocal of the absolute temperature for the G−F equilibrium.

dicated by the apparent viscosity at e.g. an actin concentration of 0.5 g/l, which is usually 1 mPa · s. At low shear rates however, e.g. at 10^{-3} s⁻¹, the apparent viscosity measures more than 10 000 mPa · s since the structure of the fluid is essentially unhurt [11].

Conclusions

Pork liver actin can be prepared in a state of highest purity by reducing the high viscosity levels in pork liver actin preparations, a factor which normally prevents the separation of the actin from actin-associated proteins during the isolation procedure. By the experimental procedure described, most of the actin-associated proteins, containing relatively small quantities of actin, were pelleted. At the same time there was a marked decrease in viscosity. This was achieved by gradually varying the pH of the actin-enriched solution, over a time period of about 3−5 h, in a specific buffer, generally containing about 5 g/l of total protein.

Under these controlled conditions most of the actin remains in the supernatant and can be concentrated and separated as described above.
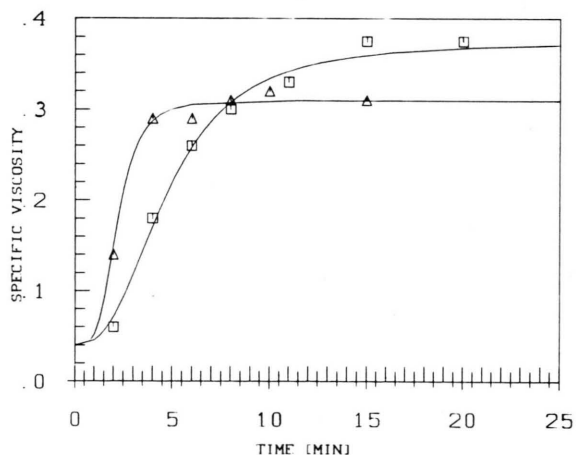

Fig. 4. Polymerization of liver actin. Viscosity-time-plot of polymerization of 8.8 µM pork liver G-actin in 2 mM Tris/HCl, 0.5 mM ATP, 0.08 mM $CaCl_2 \cdot 2H_2O$, 0.1 mM DTE (pH 7.5) with either 2 mM Mg^{2+} ions △ or 100 mM KCl □ at 298 K.

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