ISOLATION AND IDENTIFICATION OF AN ACTIN FROM RAT LIVER

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1. Introduction

Phalloidin, a toxic bicyclic heptapeptide from poisonous *Amanita* mushrooms [1] leads to a markedly enhanced appearance of microfilaments in membrane preparations from livers of rats poisoned in vivo as well as in vitro [2]. Morphologically and by their reaction with heavy meromyosin under formation of arrowhead-like structures, the filaments are indistinguishable from actin filaments of rabbit muscle [3,4]. Although these properties strongly point to an actin-like nature, it was desirable to isolate and to characterize the liver filaments by additional comparing experiments. A characteristic property of actin recently described is its inhibitory effect on DNase-I [5], which can be counteracted by phalloidin [6]. We have now isolated a protein from cytoplasma membranes of rat liver by affinity chromatography on DNase-agarose [5] and identified it with muscle actin by gel electrophoresis and by its property to inhibit DNase-I by concentrations comparable with those of muscle actin. Furthermore the phalloidin containing filaments from livers of poisoned rats were isolated by gel chromatography and identified as actin like filaments by electron microscopy.

2. Materials and methods

G-actin, prepared from rabbit skeletal muscle and free from troponin, tropomyosin and myosin was a gift from Dr H. D. Mannherz, Max-Planck-Institut für medizinische Forschung, Heidelberg. DNA purchased from Merck-Schuchardt, DNase-I from Boehringer Mannheim and Biogel A-15 from Bio-Rad, Richmond, California, were commercial products.

Membranes of 8 rat livers were prepared following the method of Ray [7] and purified on a linear 30–45% sucrose gradient. After washing the light membrane free of sucrose with twice-distilled water, proteins were extracted with EDTA according to Marchesi et al. [8]. The extract was lyophilized and the protein purified on a column of DNase-agarose according to the method of Lazarides and Lindberg [5]. Parallely membrane proteins were extracted with EDTA from the membrane fraction obtained as above [7] but from livers of rats poisoned [^{3}H] desmethylphalloin [9] (2 mg per kg, spec. act. 32 μCi/mg) 20 min before sacrifice. After dialysis of this extract against twice-distilled water and lyophilization, it was chromatographed on a Sephadex G-200 column as described by Marchesi et al. [8]. Radioactivity was measured in a Packard Tricarb Scintillation Counter.

3. Results and discussion

The membrane fraction of 8 rat livers (40 g wet weight) yielded by extraction with EDTA and lyophilization [8] 5 mg proteins which gave 1 mg of a protein fraction by affinity chromatography on elution in presence of 3 M guanidine–HCl (peak V in

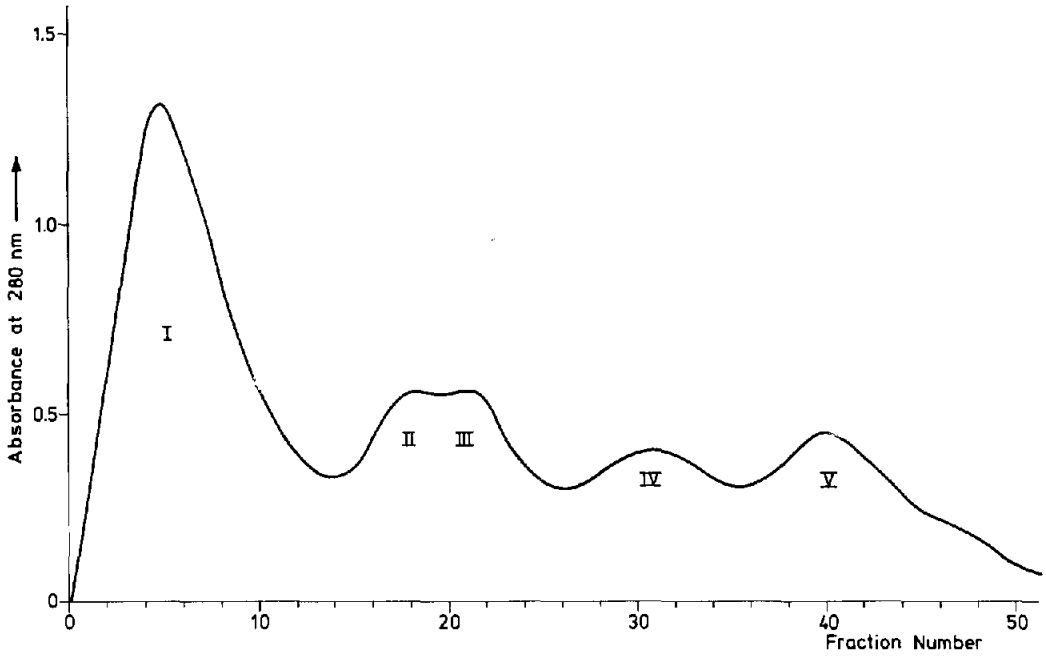

Fig.1. Affinity chromatography on DNase-agarose of EDTA extracts from rat liver plasma membranes dialysed against bidistilled water and lyophilized, suspended and eluted, collecting 3 ml fractions, in 30 ml of 0.15 M NaCl, 50 mM Tris–HCl, 1 mM $CaCl_2$ (pH 7.4): (I), elution with 30 ml 0.5 M sodium acetate, 1 mM $CaCl_2$, 30% glycerol (buffer A): II + III; with buffer A + 30 ml 0.75 M guanidine-hydrochloride: IV and with buffer A + 30 ml 3 M guanidine-hydrochloride: V.

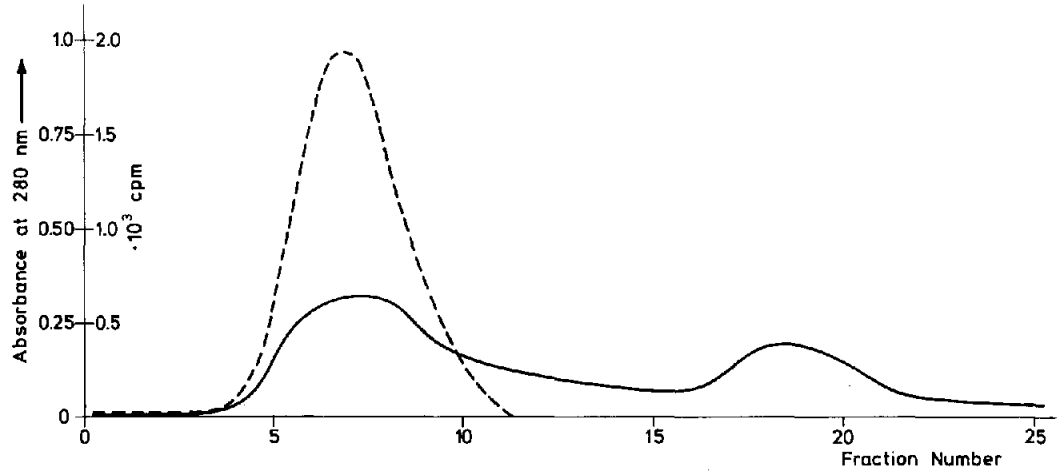

Fig.2. Gelfiltration of EDTA extracted membrane proteins on Sephadex G-200 (2.5 × 100 cm) equilibrated with 25 mM Tris, 1 mM $CaCl_2$ and 5 mM β-mercaptoethanol (pH 8.5). Radioactivity (—) was measured in each fraction of 3 ml.

fig.1) and subsequent dialysis against twice-distilled water.

The protein of peak V showed one single band in SDS gel electrophoresis which migrated like muscle actin corresponding to a mol. wt of 42 000 ‹ Its inhibitory effect on DNase-I agreed satisfactorily well with that of actin: 50% inhibition was exerted at a ratio of 1.7 mol of liver protein per mole of enzyme as compared with 2.2 mol of muscle actin. The inhibitory effect of liver protein could be counteracted by phalloidin in a way very similar to that of muscle actin [6].

The EDTA extract of the membrane fraction of livers of rats intoxicated with [^{3}H] desmethylphalloin was separated by gel chromatography on Sephadex G-200. The diagram of fig.2 shows 2 fractions of high and low molecular weight respectively. The radioactive phallotoxin is co-eluted with the first fraction in which it is tightly bound to the protein, thus stabilizing the filaments observable by electron microscopy.

The above results confirm the conclusion that filaments observed after phalloidin treatment in the isolated plasma membrane fraction in vivo or in vitro [2] consist of actin which must be situated close to the membrane or could even be an integral protein of the plasma membrane of hepatocytes. Its total identity with muscle actin of the same species remains to be elucidated by amino acid analysis and fingerprint mapping.

Acknowledgement

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