ISOLATION AND CHARACTERIZATION OF ACTIN-LIKE PROTEIN FROM YEAST *SACCHAROMYCES CEREVISIAE*

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1. Introduction

The cytoplasm of eukaryotic cells contains 5–7 nm wide microfilaments identified for 60 cell types as actin filaments by their specific ability to bind heavy meromyosin [1–4]. Actin has been isolated from > 15 cell types [4]. It accounts for 20–30% of the total protein in actively motile cells such as amoebae or blood platelets and 1–2% of total protein in mammalian tissues [1,4]. Nonmuscle actins are very similar to each other and by their properties closely resemble rabbit skeletal muscle actin [1–4]. Molecular weights of all actin-like proteins so far studied are close to 42 000; each actin molecule binds one molecule of adenine nucleotide; the amino acid composition of actins from various sources is similar; under specific conditions actins polymerize and form F-actin which is able to activate Mg^{2+}-dependent myosin ATPase [1–4]. In addition, various actins share another common feature namely the ability to inhibit DNase I [5]. It is thought that in nonmuscle cells actin-like proteins may be part of systems responsible for amoeboid motility, phagocytosis, cell division and other types of motility processes [1–3].

To investigate the role of actin in cellular functions we chose as the object yeast *Saccharomyces cerevisiae*. The selection of yeast as the object for this type of studies seems promising for several reasons: yeast is the simplest eukaryotic organism; one can easily obtain large amounts of homogeneous and if necessary synchronized cells; the technique of mutagenesis and genetic manipulations for yeast are now very advanced.

Here we demonstrate that actin-like protein can be isolated from the yeast, *Saccharomyces cerevisiae*. It is mol. wt ~42 000; it polymerizes into 7 nm wide filaments; binds one molecule of adenine nucleotide/molecule of protein; inhibits DNase I and activates Mg^{2+}-dependent ATPase of rabbit skeletal muscle myosin.

2. Materials and methods

A haploid strain of baker's yeast *Saccharomyces cerevisiae* 3P-2I9 (*ade I-I4 his x lys 2-A-I2*) from Peterhoff genetic stock was grown aerobically and harvested as in [6]. Cells (50 g) were suspended in buffer I containing 3 mM of imidazole, 0.1 mM $CaCl_2$, 0.5 mM ATP, 0.1 mM dithiothreitol (DTT), $pH_{20°C}$ 7.5 and shaken in a Braun disintegrator with 0.5 mm glass beads for 1 min. Homogenate was centrifuged at 100 000 × *g* for 90 min, pellet discarded and supernatant (extract) used for actin isolation.

Actin was isolated from yeast extract according to [7] using DEAE-cellulose saturated with ATP. Details of isolation procedure are given in the legend to fig.1.

Polyacrylamide gel electrophoresis of proteins in the presence of 0.1% sodium dodecylsulphate (SDS) was by the Laemmli method [8].

DNase I activity was measured at 20°C by hyperchromic effect [9] using pancreatic DNase I (Sigma DN-100) and calf thymus DNA (Sigma D1501).

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For measurements of ATPase activity of rabbit skeletal muscle myosin release of P_i was followed according to the Panusz modification of the Delsal procedure [10]

Rabbit skeletal muscle actin was purified from acetone powder [11]

For electron microscopy actin samples were negatively stained with 1% uranyl acetate [12] and examined in a Philips EM201C microscope at 60 kV

F-actin-bound adenine nucleotide was determined after removal of exogeneous ATP using Dowex I-X4-Cl and subsequent extraction of protein bound ADP into perchloric acid [7] Concentration of free nucleotide was measured spectrophotometrically at 257 nm

Protein concentration was determined spectrophotometrically using the following extinction coefficients ($E^{1\%}_{280}$) 5 6 for myosin, 11 0 for actin, and 12 3 for DNase I

3 Results

3 1 *Isolation of actin-like protein from* Saccharomyces cerevisiae

Figure 2b shows the results of SDS -polyacrylamide electrophoretic analysis of crude homogenate obtained by disruption of *Saccharomyces cerevisiae* cells It can be seen that the homogenate contains a protein fraction with the same electrophoretic mobility as rabbit skeletal muscle actin (fig 2a)

To purify the actin-like protein the homogenate was centrifuged at 100 000 × *g* and supernatant (extract) chromatographed on DEAE-cellulose saturated with ATP [7] The profile of the elution is shown in fig 1 Fractions obtained were tested for the presence of DNase I inhibiting activity, it was found to be present in the fractions eluted between 0 19–0 22 M KCl According to [13] at this ionic strength actin-like proteins of nonmuscle cells are eluted from DEAE-cellulose Fractions containing DNase I inhibitor activity (hatched area fig 1) were pooled, concentrated by ultrafiltration on Amicon PM-10 membrane and purified further by polymerization For this, Mg^{2+} was adjusted to 2 mM and the solution incubated at 25°C for 60 min The solution was then dialyzed overnight against buffer III containing 5 mM imidazole, 2 mM $MgCl_2$, 50 mM KCl, 0 5 mM ATP, 0 02% NaN_3

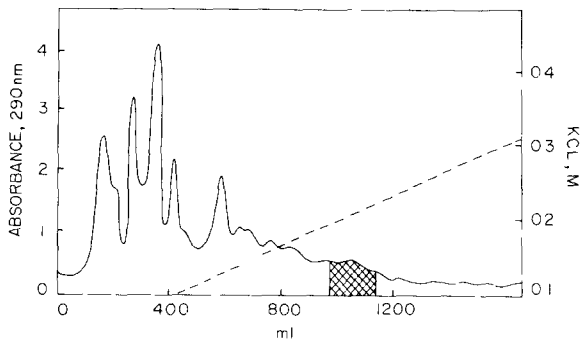

Fig 1 DEAE-cellulose chromatography of *Saccharomyces cerevisiae* extract Extract 150 ml was applied to a DEAE-cellulose column (Whatman DE52, column size 50 × 2 5 cm) Column was pre-equilibrated with buffer II (10 mM imidazole, 0 1 mM $CaCl_2$, 0 5 mM ATP, 0 2 mM DTT, $pH_{20°C}$ 7 5) containing 0 1 M KCl and saturated with ATP [7]. After application of sample, the column was washed with 200 ml buffer II containing 0 1 M KCl and elution was carried out using 2 l linear 0 1 – 0 5 M KCl gradient on buffer II at 50 ml/h flow rate The elution of protein was monitored by measurement of $E_{290\ nm}$ to decrease ATP interference during measurements All chromatography steps were done at 4°C Hatched area corresponds to the fractions where actin-like protein was found as judged by the inhibition of DNase I

(pH 7 5) F-actin was collected by centrifugation at 100 000 × *g* for 3 h The F-actin precipitate was dissolved in 2 ml buffer III As shown by electrophoretic analysis, the protein preparation is > 90% homogeneous and has the same electrophoretic mobility in SDS–polyacrylamide gels as rabbit skeletal muscle actin (fig 2c,d)

3 2 *Characterization of actin-like protein isolated from* Saccharomyces cerevisiae

The protein isolated from *Saccharomyces cerevisiae* described above reveals certain features which are specific for rabbit skeletal muscle actin

(1) Polymerization

One of the important properties of actin is the ability to form under certain conditions (0 1 M KCl, 2 mM $MgCl_2$ and the presence of ATP) long fiber-like polymer structures (filaments) which are easily identified by electron microscopy Electron microscopy of yeast actin-like protein under conditions of polymerization was carried out and the results are presented in fig 3 It is seen that this protein is able to polymerize forming long

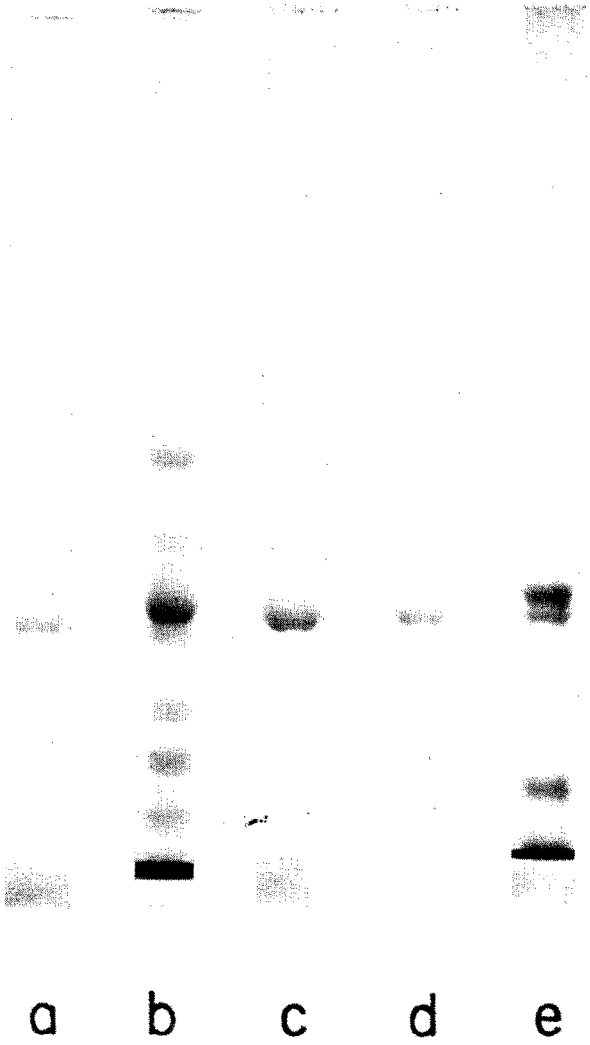

Fig.2. 7.5% polyacrylamide–0.1% SDS–gel electrophoresis of various samples: (a) rabbit skeletal muscle actin; (b) *Saccharomyces cerevisiae* supernatant after centrifugation of yeast homogenate at 20 000 × *g* for 20 min; (c) purified actin-like protein from *Saccharomyces cerevisiae*; (d) combined electrophoresis of yeast actin-like protein and muscle actin; (e) combined electrophoresis of yeast actin-like protein and yeast extract (100 000 × *g* supernatant).

fiber-like structures which are 7 nm wide. Each filament has a double supercoil structure characterized by the same parameters as are known for F-actin isolated from skeletal muscle.

(ii) Binding of adenine nucleotide

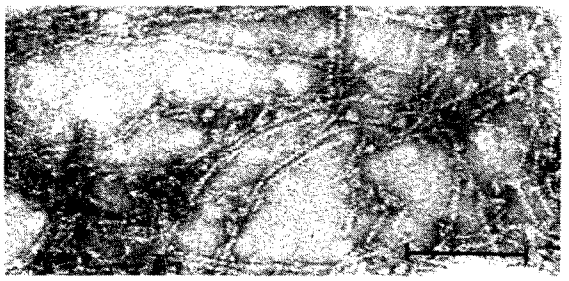

Fig.3. Electron micrograph of yeast actin-like protein sample negatively stained with 1% uranyl acetate; bar = 100 nm.

Native muscle actin always contains 1 mol bound adenine nucleotide/mol protein (monomer). It was found that yeast actin-like protein also contains ~1 mol of adenine nucleotide (characteristic absorbtion spectrum in the ultraviolet region was obtained) per 1 mol of protein monomer, i.e., actin-like yeast protein has 0.82 mol and rabbit skeletal muscle actin 0.85 mol adenine nucleotide/mol protein.

(iii) Inhibition of DNase I

Muscle actin is known to inhibit DNase I and forms stoichiometric complex with this enzyme with the ratio of actin:DNase I = 1:1 and $K_{ass} = 10^{10}$ M^{-1} [5,14]. Figure 4 demonstrates the kinetics of DNase reaction in the presence of various amounts of actin-like yeast protein. It is seen that yeast actin-like protein inhibits DNase I and even small (on molar basis) excess of this protein completely inhibits the enzyme. This fact suggests that yeast actin-like protein can form stable complex with DNase I.

(iv) Activation of myosin Mg^{2+}-dependent ATPase

The ability of yeast actin-like protein to interact with myosin was tested by its ability to activate Mg^{2+}-dependent ATPase from rabbit skeletal muscle. As table 1 shows actin-like protein activates Mg^{2+}-dependent myosin ATPase > 4-fold under these experimental conditions. This fact demonstrates that actin-like protein is capable to interact with muscle myosin.

Thus, highly purified protein from yeast with mol. wt ~42 000 posesses all characteristics known for muscle actin and therefore can be termed 'actin-like'.

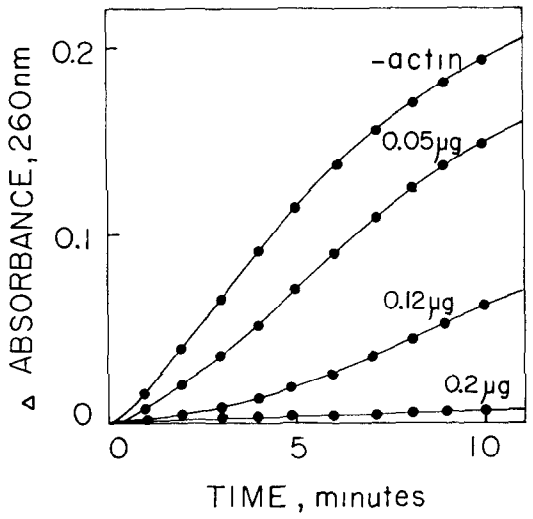

Fig 4 DNase I inhibition by yeast actin-like protein To 1 ml of DNA solution in buffer (100 mM Tris, 4 mM $MgCl_2$, 1 7 mM $CaCl_2$, pH 7 5) 0 1 mg DNase I was added followed by 0 05–0 2 mg yeast actin-like protein in 20 ml solution The sample was stirred for 10 s and hyperchromic effect registered The absorbance due to DNA was equal to 0 9 A_{260}/ml and was equilibrated with control sample

4. Conclusion

This study demonstrates that a protein can be isolated from *Saccharomyces cerevisiae* with the properties characteristic for muscle actin This protein has mol wt ~42 000, it polymerizes with the formation of characteristic 7 nm wide, long filaments, it binds one molecule of adenine nucleotide per monomer of protein, it inhibits DNase I with the formation of a stable complex, it is capable interacting with muscle myosin activating its Mg^{2+}-dependent ATPase All these facts strongly suggest that the simplest eukaryotic cells *Saccharomyces cerevisiae* contain actin-like protein By analogy with higher eukaryotes it can be suggested that the actin-like protein in yeast may function in various cytokinetic processes

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Table 1
Activation of Mg^{2+}-dependent myosin ATPase by yeast actin-like protein[a]

Components for ATPase assay	ATPase spec act (nmol P_i/min^{-1} mg myosin^{-1})
Actin-like protein	< 1
Muscle myosin	7 5
Actin-like protein + muscle myosin	34

[a] Reaction mixture (0 5 ml) contained 10 mM imidazole, 2 5 mM $MgCl_2$, 0 2 mM DTT, 0 5 mM EGTA, 0 5 mM ATP, 0 2 mg/ml rabbit skeletal muscle myosin and 0 2 mg/ml actin-like protein, $pH_{20°C}$ 7 0 Incubation, 10 min at 37°C

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