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On an Mg^{2+}-dependent interaction of actin with glyceraldehyde-phosphate dehydrogenase

The fundamental role of KCl in the organization of F-actin

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In the presence of Mg^{2+}, the formation of actin filaments is hindered by glyceraldehyde-3-phosphate dehydrogenase. This effect, which increases with the square of Mg^{2+} concentration, is counteracted by 0.15 M KCl. Thus KCl, at concentrations found in the intracellular compartment, appears to be strictly required for the correct formation of actin filaments in all tissues in which the glyceraldehyde-phosphate dehydrogenase concentration is high.

Mg^{2+}; Glyceraldehyde-phosphate dehydrogenase; Actin filament organization

1. INTRODUCTION

The structural and functional implications of the interactions of glyceraldehyde-phosphate dehydrogenase (D-glyceraldehyde-3-phosphate:NAD^+ oxireductase (phosphorylating), EC 1.2.1.12) (GAPD) with regulated as well as non-regulated actin filaments have been widely investigated [1–8]. GAPD was found to interact with the actin cytoskeleton in fibroblasts [9] and to follow the distribution of dispersed actin when the cytoskeleton is disrupted by exposure of the cells to 2-deoxyglucose [10].

We describe here an Mg^{2+}-dependent interaction of GAPD with actin and discuss its possible physiological significance. GAPD was in fact found to hinder the formation of actin filaments and to favour that of amorphous aggregates. The rate of formation of the aggregates was found to be proportional to the square of the Mg^{2+} concentration. KCl was found to counteract this phenomenon.

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2. MATERIALS AND METHODS

G-Actin from rabbit muscle was prepared according to Spudich and Watt [11] and further gel filtered through Sephadex G-150 [12]. The actin concentration was measured from the absorbance at 290 nm, the absorbance of 1 mg pure actin/ml (light path 1 cm) being taken to be 0.62 [13]. The molar concentration of G-actin was calculated on the basis of a molecular mass of 42 kDa [14]. GAPD from rabbit muscle (spec. act. 80 IU/mg protein) was from Boehringer (Mannheim). The crystalline suspensions were sedimented by centrifugation, dissolved in 1 mM EDTA, 1 mM mercaptoethanol, pH 7.0, and dialyzed overnight at 2°C against the same solution. The dehydrogenase concentration was measured from the absorbance at 280 nm, the absorbance of 1 mg/ml pure enzyme per ml (light path 1 cm) being taken to be 1.02 [15]. The molar concentration was calculated on the basis of a molecular mass of 145 kDa [16]. In the experiments freshly dialyzed solutions of GAPD (24 h) were used.

For electron-microscopic observations, one drop of the protein solutions was diluted with 9 drops of the suspension buffers; one drop of the diluted

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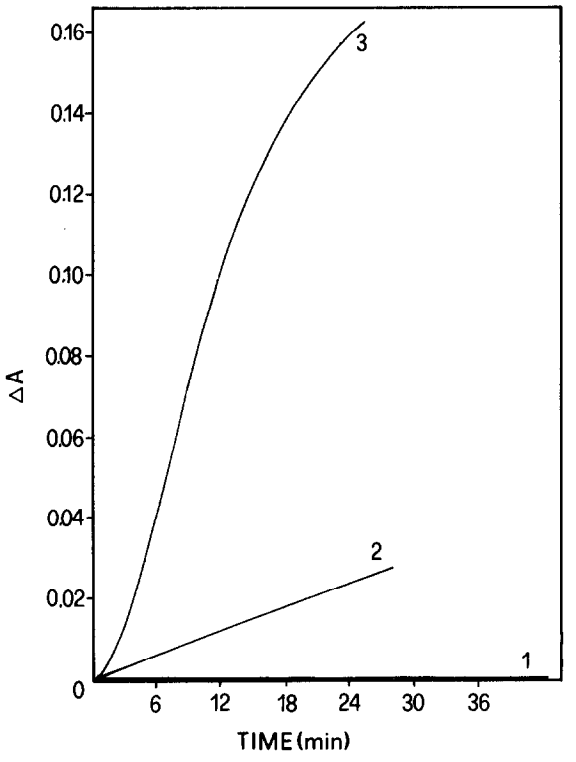

Fig.1. Turbidimetric study of the interaction between actin and GAPD. Incubation mixtures contained 6 μM ATP–G-actin, 3 μM GAPD, 20 μM ATP, 20 μM $CaCl_2$, 50 μM 2-mercaptoethanol, 100 μM NaN_3 and 5 mM Tris-HCl buffer (28°C, pH 7.35). Control sample (1), 2 mM $CaCl_2$ added (2), 2 mM $MgCl_2$ added (3). The increase in turbidity was followed at 400 nm.

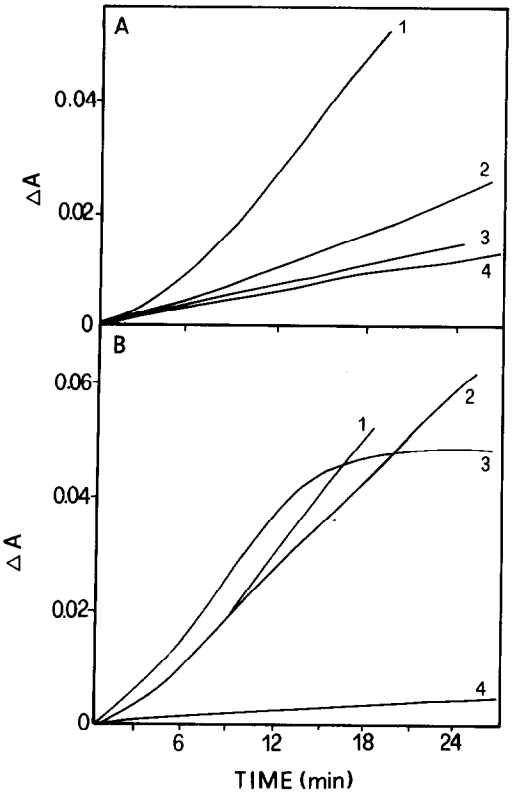

Fig.2. Interaction between ATP–G-actin and GAPD as a function of the concentration of the two proteins. (A) Mixtures contained 3 μM GAPD, 10 μM ATP, 10 μM $CaCl_2$, 50 μM 2-mercaptoethanol, 100 μM NaN_3, 5 mM Tris-HCl buffer and either 6 μM (1), 4 μM (2), 2 μM (3) or 1 μM ATP–G-actin (4) (28°C, pH 7.35). The increase in turbidity was followed at 400 nm. (B) Mixtures contained 6 μM ATP–G-actin and either 3 μM (1), 1.5 μM (2), 0.75 μM (3) or 0.3 μM (4) GAPD. Other conditions as in (A).

solution was immediately applied to a carbon-coated 400-mesh grid and, after 60 s, stained with 5 drops of 1% (w/v) uranyl acetate, pH 4.25. Electron micrographs were taken on a Hitachi H-800 electron microscope.

3. RESULTS AND DISCUSSION

In the presence of 2 mM $MgCl_2$, 6 μM ATP–G-actin and 3 μM GAPD interact with the formation of turbidity. After an initial lag phase, the increase in turbidity takes place at a constant rate for many minutes (fig.1). The rate of this phase is proportional to the square of the $MgCl_2$ concentration (not shown). In the presence of 2 mM $CaCl_2$ turbidity formation is much less evident (fig.1). The rate of turbidity increase and the kinetics of the phenomenon depend not only on the concentration of actin and GAPD but also on their molar ratios (fig.2A,B). In particular, with 6 μM ATP–G-actin and 0.75 μM GAPD, the turbidity rapidly reaches a plateau (fig.2B, trace 3), a likely expression of the formation of a different supramolecular structure. This view is confirmed by electron microscopy. With 6 μM ATP–G-actin and 3 μM GAPD, in the presence of 2 mM $MgCl_2$, only large, amorphous aggregates are formed (not shown). In contrast, with 0.75 μM GAPD, 6 μM

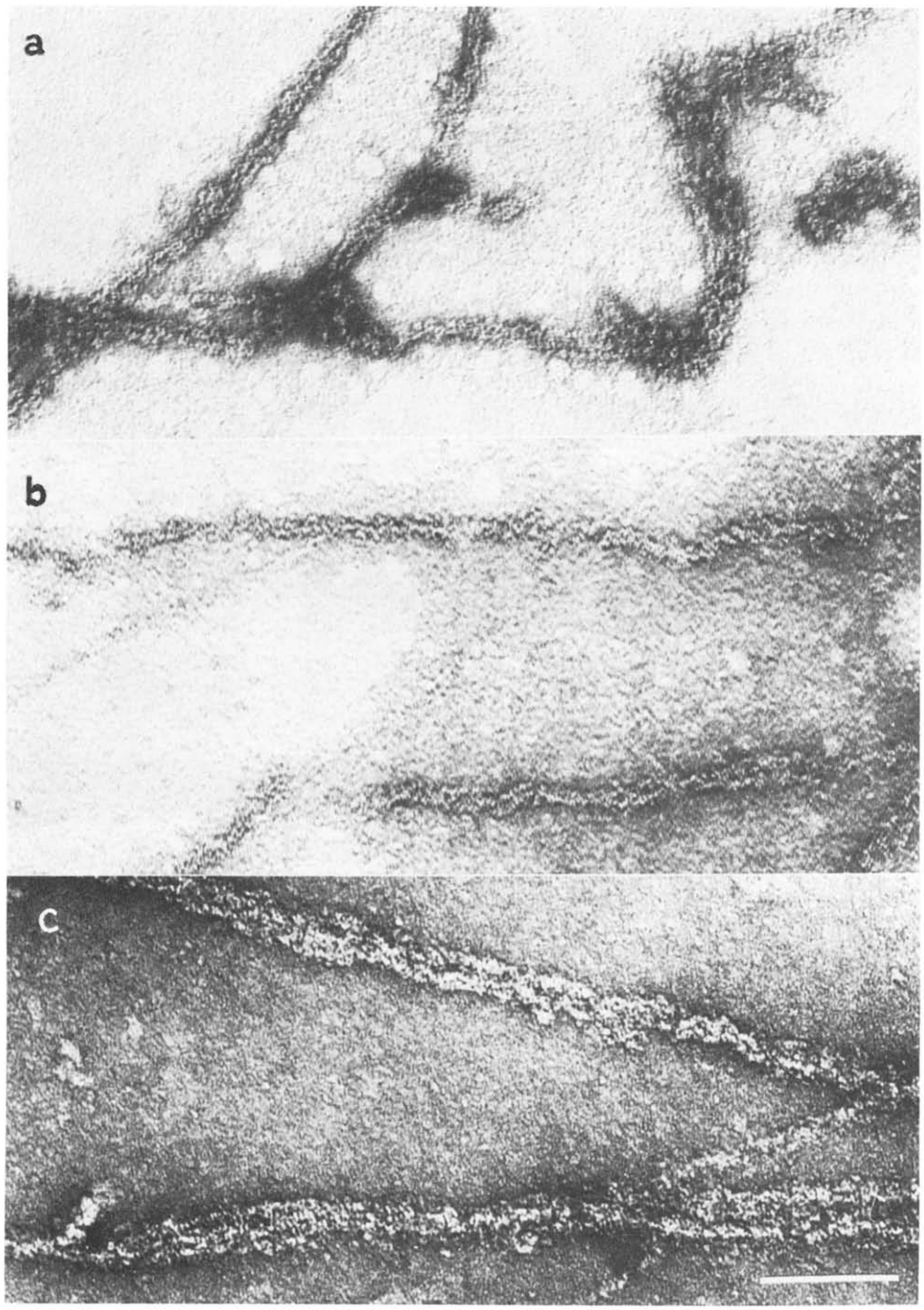

Fig.3. Electron micrographs of mixtures of actin and GAPD in the presence of $MgCl_2$. (A) 6 μM ATP–G-actin, 0.75 μM GAPD, 1 mM $MgCl_2$; (B) 6 μM ATP–G-actin, 3 μM GAPD, 2 mM $MgCl_2$, 1 mM ATP, 0.15 M KCl; (C) F-actin (6 μM as the monomer, polymerized in the presence of 2 mM $MgCl_2$), 3 μM GAPD, 2 mM $MgCl_2$. All samples were incubated for 20 min at 20°C and pH 7.35 before being applied to the grids. Bar, 125 nm.

ATP–G-actin still assembles into bundles of filaments (fig.3A).

Of interest is the observation that 0.15 M KCl allows the formation of filament bundles from 6 μM ATP–G-actin even in the presence of 3 μM GAPD and 2 mM $MgCl_2$ (fig.3B). These bundles are similar to those formed starting from F-actin (fig.3C).

We have thus shown that, in the presence of $MgCl_2$ as the only polymerizing agent, GAPD seriously interferes with the formation of actin filaments. In fact, at 1 mM $MgCl_2$, no filaments are formed from 6 μM ATP–G-actin when the concentration of GAPD exceed 0.75 μM. White rabbit muscle contains about 600 nmol actin and 100 nmol GAPD per g fresh tissue [17]. The ratio between the two proteins is about the same as the maximum ratio compatible with the organization of actin into filaments in the presence of 1 mM $MgCl_2$. However, since the $MgCl_2$ concentration in muscle is greater than 1 mM and the rate of the interaction of actin with GAPD is directly proportional to the square of the $MgCl_2$ concentration, actin filament organization would certainly be hindered. Additional difficulties could be encountered in the course of myoblast fusion in which a higher GAPD/actin molar ratio is reported to occur [18,19]. Under these conditions, therefore, a high concentration of KCl, such as that found in the intracellular compartment, appears to be strictly required for the correct polymerization of actin in the presence of GAPD. Possibly KCl may be strictly required for the reversible microfilament assembly in other cell types as well.

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