VERSATILITY OF G-ACTIN AS THE BUILDING BLOCK OF BIOLOGICAL STRUCTURES

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1. Introduction

Binding of glycolytic enzymes to structure protein of the muscle has been widely described [1–5]. As an example, the interaction of F-actin with aldolase, occurs with the ratio of 1 molecule of aldolase to 2 G-actin monomers of the F-actin chain [3] and is accompanied by modification of the kinetic parameters of aldolase [5].

We describe here a new kind of interaction between G-actin monomer and aldolase. As a result of this interaction, for each aldolase subunit present in the solution, 1 molecule of G-ATP actin is converted into G-ADP actin and a gel is formed. The formation of the gel requires the availability of the active site as well as of an allosteric site of the aldolase subunits; dephosphorylation of G-ATP actin requires only the availability of the allosteric site.

2. Experimental

For the experiments described below rabbit muscle aldolase (spec. act. 16 units/mg protein) was prepared as in [6] and rabbit muscle actin as in [7].

Glyceraldehyde 3-phosphate dehydrogenase from lobster muscle was kindly supplied by Professor G. L. Rossi, University of Parma. The inactive glycerolphosphate derivative of aldolase (spec. act. 0.025 unit/mg protein) was prepared as in [8].

3. Results and discussion

At low ionic strength muscle G-ATP actin remains in a monomeric state (mol. wt 48 000) and does not polymerise nor release orthophosphate from the bound ATP. When 50 μM G-ATP actin and 25.2 μM aldolase subunit are mixed, inorganic orthophosphate is produced (fig.1). The release of orthophosphate occurs in a rapid and in a slow phase. The concentration of orthophosphate produced in the rapid phase (22.5 μM), as measured by linear extrapolation to the

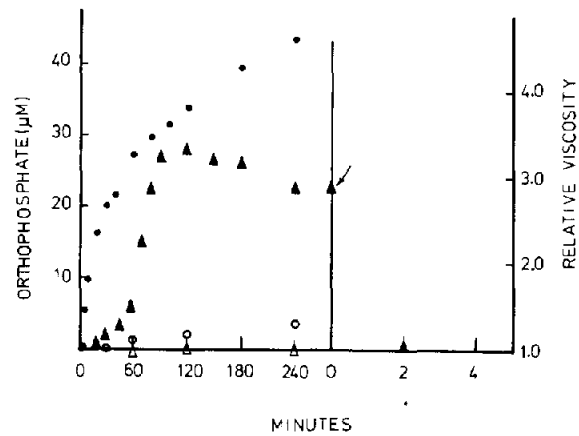

Fig.1. The interaction of aldolase and G-actin. The incubation mixtures contained: 50 μM G-ATP actin; 25 μM aldolase subunit; 20 μM ATP; 20 μM $CaCl_2$; 2 mM Tris–HCl, pH 7.3. Temp. 23°C. At the time indicated by the arrow (240 min incubation) either 0.5 mM fructose bisphosphate or 0.5 mM hexitol bisphosphate or 0.5 mM ATP was added and viscosity measured after 2 min. At timed intervals samples of the incubation mixture were treated with 0.15 M trichloroacetic acid, protein was removed by centrifugation and orthophosphate was determined as in [10]. Aliquots, 1 ml incubation mixtures, were introduced into an Ostwald viscosimeter maintained at 23 ± 0.1°C and the flow time for the sample measured at intervals. G-actin + aldolase: (●) Orthophosphate formed; (▲) relative viscosity. G-actin alone: (○) Orthophosphate formed; (△) relative viscosity.

ordinate axis of the first part of the slow phase, corresponds to the aldolase subunit concentration.

In similar experiments, in which 50 μM G-actin was mixed with different concentrations of aldolase subunit ranging from 10–25 μM, the concentration of orthophosphate produced in the rapid phase was always equal to the concentration of the aldolase subunit added in every single experiment.

In the low ionic strength solution actin or aldolase alone have low and constant viscosity but on mixing the viscosity increases slowly at the beginning and then rises sharply to a constant value (fig.1).

The sudden increase of the viscosity is concomitant with the end of the rapid phase of the orthophosphate production. Viscosity is restored to the low initial value by the addition of either 0.5 mM fructose bisphosphate or 0.5 mM hexitol bisphosphate, a competitive inhibitor of aldolase [9], or 0.5 mM ATP (fig.1).

When aldolase is mixed with G-actin in the presence of hexitol bisphosphate or when the inactive glycerolphosphate derivative of aldolase (in which the active site is irreversibly blocked) is mixed with G-actin, the production of orthophosphate is not followed by the increase of the viscosity and by the formation of the gel (table 1).

Since gelation requires that G-actin acts as a bridge between 2 subunits of 2 different aldolase molecules, it is suggested that G-actin interacts with the active site of a 1st aldolase subunit and with an allosteric site of a 2nd aldolase subunit. It happens indeed that, when the active sites of aldolase are irreversibly blocked, as in the glycerylphosphate derivative, gelation does not occur, although orthophosphate is still produced. The last phenomenon is of interest since

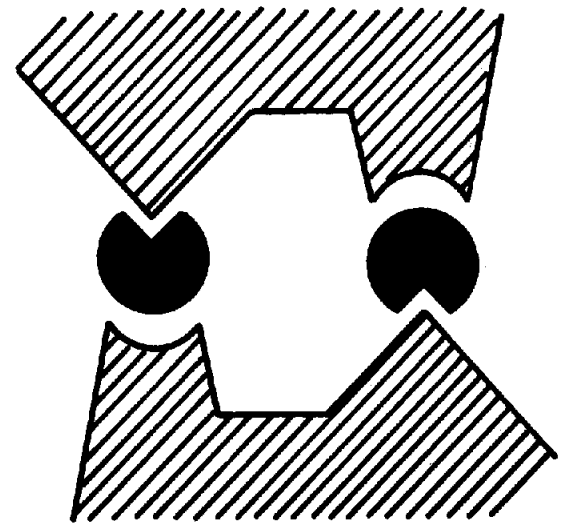

Fig.2. Two molecules of G-actin form a bridge between 2 subunits of 2 different aldolase molecules. The shaded areas represent the active and the allosteric sites of 2 aldolase subunits. The filled areas represent 2 G-actin molecules.

Table 1
The gelation of the G-actin–aldolase solution requires a free aldolase active site

	Orthophosphate formed (μM)	Relative viscosity
G-actin + native aldolase	8.6	1.7
G-actin + native aldolase + hexitol 1,6-bisphosphate	6.9	1.0
G-actin + β-glycerylphosphate aldolase derivative	4.5	1.03
G-actin	0.0	1.00

The incubation mixtures contained: 10.2 μM G-ATP actin; 25 μM subunit of either native aldolase or of its β-glycerylphosphate derivative; 20 μM ATP; 20 μM $CaCl_2$; 2 mM Tris–HCl buffer, pH 7.3. After 60 min incubation at 23°C orthophosphate production and the viscosity were measured as in fig.1

it shows that the conversion of G-ATP actin into G-ADP actin occurs also at low ionic strength and is triggered even by a single interaction with a protein different from G-actin.

A hypothesis can be formulated concerning the structure of the gel which forms as each aldolase subunit interacts with one molecule of G-actin. The stoichiometry is satisfied by a gel structure in which the subunits of 2 different aldolase molecules are connected by 2 G-actin molecules, each acting as a bridge between the active and the allosteric sites (fig.2). The slow orthophosphate production which follows the interaction of G-actin and aldolase is tentatively assigned to gel G-ADP actin exchange with medium G-ATP actin.

These phenomena are specific for aldolase. Under the same experimental conditions, glyceraldehyde 3-phosphate dehydrogenase from lobster muscle and oxy- and deoxyhemoglobin from beef do not trigger the dephosphorylation of G-ATP actin or the formation of a gel when mixed with G-actin.

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