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DOES PROTAMINE DICTATE THE DIRECTION OF GROWTH OF THE ACTIN FILAMENTS?

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SUMMARY By interaction with μM protamine, at 0.1 μM free Mg^{2+} concentration, G-ATP actin dephosphorylates rapidly the* ATP added to the medium, through the cyclic conversion into G-ADP actin. As a result the stationary concentration of G-ATP actin decreases in favour of G-ADP actin. This influences the modalities of actin polymerization which may follow the "head to tail" mechanism only starting from G-ATP actin, while starting from G-ADP actin must follow either the bipolar or the unipolar polymerization mechanisms.

G-actin, at low Mg^{2+} concentration, is induced to work ciclically, like an ATPase, by the addition of 2 μM protamine. Under these conditions continuous splitting of added ATP occurs at a rate even larger of that obtained with F-actin under sonic vibrations (1, 2). The main effect of this hydrolytic activity is to decrease the steady state concentration of G-ATP actin in favour of G-ADP actin and thus to influence the modality of the polymerization of actin. It is infact known (3) that "head to tail" polymerization occurs only starting from G-ATP actin, while it is forbidden starting from G-ADP actin.

MATERIALS AND METHODS Rabbit muscle G-ATP actin was prepared according to the method of Spudich and Watt (4). Actin concentration was measured from the absorbance at 290 nm considering that the absorbance of 1 mg of pure actin/ml (light path 1 cm) is 0.62 (5). Molar concentration of G-actin

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was calculated on the basis of a molecular weight of 48,000 (6). Protamine sulfate from salmon (molecular weight 9,640) was purchased from Sigma Chemical Co. Inorganic orthophosphate production was determined by the method of Tashima and Yoshimura (7) after precipitation of the protein with 0.1 M trichloroacetic acid.

Free Mg^{++} and Ca^{++} concentrations were calculated from a log equilibrium formation constant for the 1:1 chelate of 8.69 and 10.6 respectively (8, 9) which refer to the most strongly basic form of EDTA (pK_4 and pK_3 of EDTA are 10.26 and 6.16 respectively)(8, 9). The contribution of ATP and of G-actin were ignored due to the lower association constant ($10^5 M^{-1}$) of the ATP^{4-} -Mg^{2+} complex (10) and to the low concentration of G-actin.

RESULTS

The addition of 2 μM protamine to a solution containing G-ATP actin, 20 μM $CaCl_2$, 50 μM $MgCl_2$, 0.2 mM ATP and 1 mM EDTA induces the actin to hydrolyse rapidly the ATP added to the solution. The hydrolytic reaction takes place in two phases. In the first phase inorganic orthophosphate is rapidly produced with the apparent first order rate constant of 0.046 s^{-1} at 5 μM G-actin and 2 μM protamine and of 0.02 s^{-1} at 10 μM G-actin and 2 μM protamine (Fig.1 A). In the second, stationary phase, the rate of ATP hydrolysis is 0.1 to 0.2 nmol per min per nmol of actin (Fig.1 B), that is of the same order of magnitude of the rate obtained by sonication of F-actin in the presence of ATP (1, 2). The rate of the recycling depends of free Mg^{2+} concentration (Table I) and becomes practically constant between $1,9 \times 10^{-8}$ M and 3.6×10^{-7}M Mg^{2+}.

The interaction between protamine and actin is quite strong since it occur also at subcritical actin concentrations

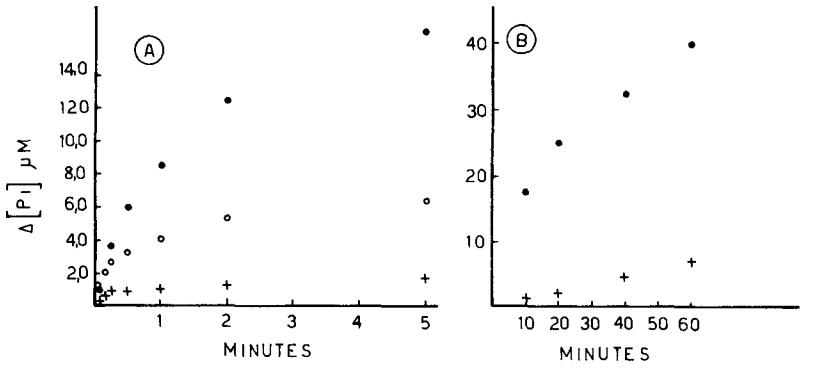

Fig.1) G-actin adenosine triphosphatase activated by protamine.

The incubation mixtures (5 ml) contained 2 mM tris-HCl buffer, 1 mM EDTA, 20 μM $CaCl_2$, 50 μM $MgCl_2$, 0.2 mM ATP and either 10 μM (•) or 5 μM (o) or 1 μM (+) G-ATP actin. After 2 min of preincubation the reaction was started by the addition of 2 μM protamine. Temperature was 22°, pH was 8.0. Control samples without protamine were also prepared.

$\Delta[Pi]$ represents the difference between orthophosphate produced in the complete system and in the control samples without protamine.

and it is not abolished by the increase of the ionic strength. This is shown in the experiments of Table II where 1 μM G-ATP actin is not dephosphorylated in the presence of 60 mM KCl while the further addition of 2 μM protamine allows the dephosphorylation. When the experiment if performed under recycling conditions (1 mM EDTA, 50 μM $MgCl_2$) one mole of inorganic orthophosphate is released per mole of G-ATP actin in the presence of 60 mM KCl alone (apparently the level of the critical concentration was decreased by the addition of EDTA because of the concomitant decrease of the free Ca^{++} concentration below 10^{-10}M), while in the presence of 60 mM

Table I

Effect of Mg^{++} on the protamine induced adenosine triphosphatase activity of G-actin.

Free Mg^{2+}	Orthophosphate produced ($nmol.ml^{-1}.min^{-1}$)
NONE	0.02
$0.3x10^{-8}$ M	0.07
$1.9x10^{-8}$ M	0.33
$4.0x10^{-8}$ M	0.54
$3.6x10^{-7}$ M	0.45

The incubation mixtures (5 ml) contained 2 mM tris-HCl 1 mM EDTA, 20 μM $CaCl_2$ either 10 ot 50 or 100 or 500 μM $MgCl_2$, 0.2 mM ATP and 10 μM G-ATP actin. A sample without Mg^{++} was also prepared. The reaction was started by the addition of 2 μM protamine. Temperature was 22°, pH was 8.0. Samples were taken in the stationary phase between the 2th and 10th min after the addition of protamine. Inorganic orthophosphate was determined and free magnesium concentration was calculated as described in the experimental section.

KCl and 2 μM protamine recycling occurs and the production of inorganic orthophosphate exceeds the amount of actin added to the system.

DISCUSSION

G-actin is induced to operate like an ATPase by μM protamine provided the free Mg^{2+} concentration is in the range of 0.1 μM. The phenomenon occurs both at low and at high ionic strength, both at subscritical and at overcritical actin concentrations. The rate of ATP hydrolysis by this

Table II

Interaction of G-actin and protamine at high ionic strength.

	Orthophosphate produced (μM)			
	10 min	20 min	40 min	60 min
Exp 1	0.0	0.0	0.0	0.0
Exp 2	0.19	0.37	0.64	1.1
Exp 3	0.4	0.6	1.2	1.2
Exp 4	1.5	2.3	3.6	5.3

In Exp 1 and 2 the incubation mixtures contained 2 mM tris-HCl buffer, 2 μM $CaCl_2$, 2 μM ATP, 60 mM KCl and 1 μM G-ATP actin.
In Exp 2, 2 μM protamine was also added. Temperature was 22°, pH was 8.0.
In exp 3 and 4 the incubation mixtures contained 2 mM tris-HCl buffer, 2 μM $CaCl_2$, 0.2 mM ATP, 60 mM KCl, 1 mM EDTA, 50 μM $MgCl_2$ and 1 μM G-ATP actin.
In exp 4, 2 μM protamine was also added. Temperature was 22°, pH was 8.0. In all the experiments the reaction was started by the addition of G-actin.
Inorganic orthophosphate was determined as described in the experimental section.

mechanism may reach 3 nmol/min/ml of citoplasmic fluid in human blood platelets where the critical concentration of actin is about 0.3 mg/ml (11).

The major biological effect of this hydrolytic activity is, however, to maintain low the steady state concentration of G-ATP actin in favour of G-ADP actin. It is known (3) that the G-actin to F-actin conversion, when starting from G-ATP actin, may occur by the "head to tail" polymerization mechanism where the actin filament lengthen at one

end and shorten at the other. With G-ADP actin, on the contrary, the "head to tail" polymerization is forbidden, the filament may grow only outwards from or inward to the centre of both ends of the aggregate. We thus propose that changes in the concentration of protamine influence the modalities of growth of the actin filaments. The "head to tail" polymerization is possible only when protamine concentration is low (and G-ATP actin is the prevalent species), bipolar or unipolar polymerizations are the only possible when protamine concentration is high (and G-ADP actin is the prevalent species). These regulation mechanisms could operate in the generation of the acrosomal process of certain echinoderm sperm (12, 13).

We have recently proposed (14) that histone and protamine trigger the formation of actin fibers running from the kinetochore of the chromosome to the pole of the mitotic spindle. With this report we have explored further the potentiality of these basic proteins as regulatory tools in the polymerization of actin.

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REFERENCES

1) Asakura S., Bioch.Bioph.Acta, 52, 65-75, 1971.
2) Dancker P. and Low I., Bioch.Bioph.Acta 484, 169-176,1977.
3) Wegner A.,J.Mol.Biol.,108, 138-150, 1976.
4) Spudich J.A. and Watt S., J.Biol.Chem.,246, 4866-4871,1971.
5) Gordon D.J., Yang Y.Z. and Korn E.D., J.Biol.Chem.,251 7474-7479, 1976.
6) Adelstein R.S., Godfrey J.E. and Kelles W.K., Bioch.Bioph. Res.Communs., 12, 34-38, 1963.
7) Tashima Y. and Yoshimura N., J.Biol.Chem.,(Tokyo) 78, 1161-1169, 1975.

8) Schwarzenbach G. and Ackerman H., Helv.Chim.Acta, 30, 1798-1804, 1947.
9) Schwarzenbach G. and Ackerman H., Helv.Chim.Acta, 31, 1029-1048, 1948.
10) O'Sullivan W.J., and Perrin D.D., Bioch.Bioph.Acta, 52, 612-614, 1961.
11) Korn E.D., Proc.Nat.Acad.Sci. USA, 75, 588-599, 1978.
12) Tilney L.G., J.Cell.Biol., 64, 289-310, 1975.
13) Tilney L.G., J.Cell. Biol., 69, 73-89, 1976.
14) Magri E., M .Zaccarini, and E.Grazi, Bioch.Bioph.Res. Communs. In the press.