PROTECTIVE ACTION OF NUCLEOSIDE TRIPHOSPHATES AGAINST THE INACTIVATION OF G-ACTIN BY ETHYLENEDIAMINETETRAACETATE*

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One of us has recently reported (Martonosi and Gouvea, 1961) that treatment of G-actin with close to equimolar concentration of ethylenediaminetetraacetate (EDTA) results in a complete loss of polymerizability with the release of the bound ATP.

This report deals with the protective effect of nucleoside triphosphates upon this inactivation.

As shown in Fig. 1, ATP in a concentration of about 10^{-3} M prevented the rapid inactivation of actin by EDTA. With lower ATP concentrations the inactivation of actin by EDTA was rather rapid and after two minutes of the EDTA treatment the addition of an excess amount of $MgCl_2$ did not induce polymerization.

The protective action of ITP against inactivation of actin by EDTA was also observed. Other structurally related compounds such as UTP, CTP and GTP were ineffective (Table 1).

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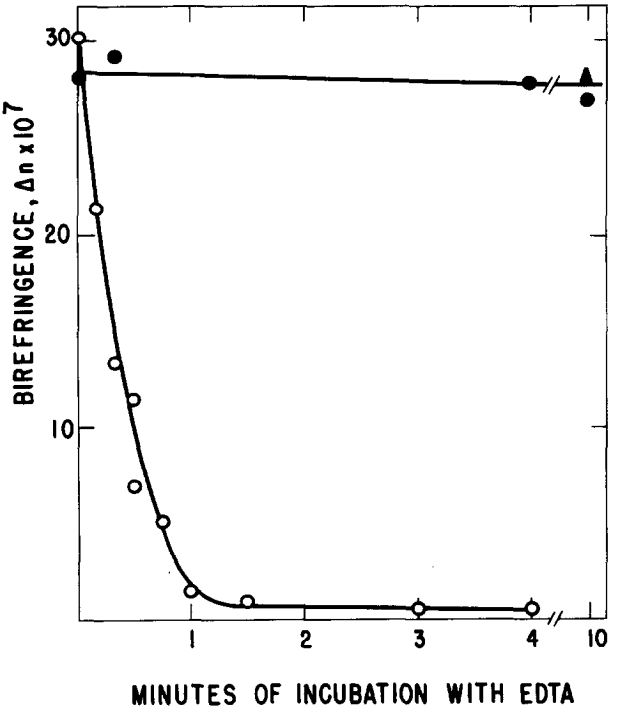

Fig. 1. Protective effect of ATP on the inactivation of G-actin by EDTA.

To a solution of G-actin, prepared as previously described (Martonosi and Gouvea, 1961) and freed of free ATP by Dowex treatment (Asakura, 1961) containing 0.35 mg of protein per ml in 0.01 M Tris buffer, pH 8.0., 22°, 0.1 mM EDTA was added at 0 time; to samples taken at various times 4 mM $MgCl_2$ was added to stop the EDTA action and to start polymerization. The total volume of the reaction mixture was 10.0 ml after addition of $MgCl_2$. Two to three hours after addition of $MgCl_2$ the birefringence of flow of the solution was measured at a velocity gradient of 1000 $sec.^{-1}$ at 20° in an Edsall type of apparatus. 0-0: without added ATP; ▲: 5×10^{-4} M ATP added before incubation with EDTA; ●-●: 10^{-3} M ATP added before incubation with EDTA.

These experiments provide further support for the view (Martonosi and Gouvea, 1961) that the protective effect of nucleoside triphosphates on G-actin require a proper configuration at the 1-2-3 positions of the purine ring.

Table 1. The protective effect of nucleosidetriphosphates against inactivation of actin by EDTA.

Nucleosidetriphosphate Added	Specific Viscosity
Control	0.67
No nucleosidetriphosphate	0.13
ATP	0.63
ITP	0.36
UTP	0.03
CTP	0.16
GTP	0.15

G-actin solutions containing 0.87 mg protein per ml, 3.0×10^{-5} M ATP, 10^{-3} M Tris buffer pH 7.8, and 3.3×10^{-5} M EDTA were incubated for 30 minutes in the presence of various nucleoside triphosphates (final concentration 5.9×10^{-4} M) at 29°. At the end of the incubation 10^{-3} M $MgCl_2$ and 0.1 M KCl were added and the polymerization of actin was followed in a viscometer. The table represents specific viscosities, 30 minutes after the addition of KCl and $MgCl_2$. No EDTA and nucleoside triphosphate were added to the control sample.

REFERENCES

1. Asakura, S., Arch. Biochem. Biophys. 92, 140, 1961.

2. Martonosi, A., Gouvea, M.A., J. Biol. Chem. 236, 1961 (In press).