INHIBITION OF ACTIN POLYMERIZATION BY MERCURIALS WITHOUT REMOVAL OF BOUND NUCLEOTIDE[1]

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Kuschinsky and Turba first reported that salyrgan-treated G-actin is unable to polymerize, and that this effect is reversed by subsequent addition of mercaptans. Barany et al (1961), Martonosi and Gouvea (1961), and Strohman (1961) have recently shown that treatment with various mercurials leads to the release of the bound ATP of G-actin. This second observation raises the question whether the involvement of SH groups is restricted to the nucleotide binding process, since ATP-free actin is unable to polymerize (Asakura, 1961), or whether certain SH groups, not necessarily identical with those required for ATP binding, are essential for polymerization. The results presented below show that SH blocking reagents can preferentially and reversibly inhibit polymerization without a corresponding loss of ATP.

When Dowex-treated G-actin containing C^{14}-ATP was treated with salyrgan the loss of polymerizability, determined with measurements of double refraction of flow (DRF) (Asakura, 1961) was essentially instantaneous. The bound nucleotide content of G-actin, which was determined by measurement of the radioactivity remaining in the solution after

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treatment with Dowex 1-X8, was decreased by only a negligible amount (Fig. 1). Prolonged incubation led to a slow loss of ATP. Under the conditions of the experiment shown in Fig. 1 this loss amounted to about 20% in 60 minutes.[4] For a considerably longer time of incuba-

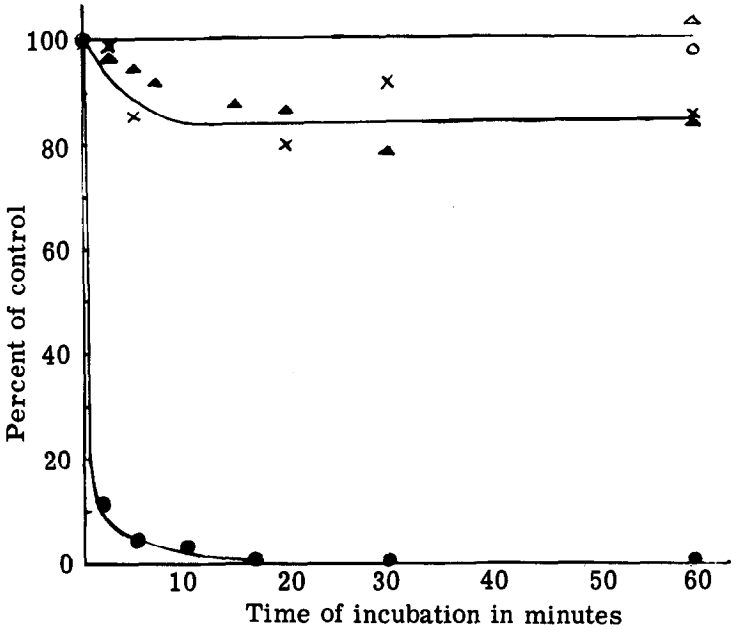

Fig. 1. Time course of salyrgan action on the polymerizability and on the bound nucleotide of G-actin.

G-actin, prepared and labelled with C^{14}-ATP as described previously (Martonosi et al., 1960), was treated with Dowex 1-X8 (Cl^- form, 200-400 mesh, 0.1 meq/ml) in order to remove free nucleotides. This actin solution, containing 3.4 mg of protein per ml, was incubated with 8.8 moles of salyrgan per mole (MW=60,000) of actin at 23° in 2 mM Tris, pH 7.0. At each time indicated on the abscissa, three samples were taken. One was polymerized by adding 0.1 M KCl and 1 mM $MgCl_2$ (final concentrations); the second was similarly polymerized but in the presence of 0.01 M glutathione; the third sample was treated with Dowex 1-X8 (0.1 meq/ml) and an aliquot, freed from the resin by centrifugation, was dried on filter paper and counted in a Packard Tri-Carb Scintillation Counter essentially according to Loftfield and Eigner (1960). The DRF of the polymerized actin was measured 15 to 20 minutes after addition of salts. Samples treated with salyrgan: ▲——▲: bound nucleotide; ●——●: DRF after polymerization without glutathione; X——X: DRF after polymerization with glutathione: Control samples (without salyrgan): △——△: bound nucleotide; ○——○: DRF after polymerization without glutathione.

4) It appears from the data of Martonosi and Gouvea (1961) that the loss of ATP following treatment with salyrgan is considerably faster when the Mg^{++} - precipitation method is used for the determination of the bound ATP.

tion, 20 to 24 hours, there was a complete release of ATP, compared to a 50% decrease in untreated actin that was also kept at room temperature.

Figure 2 shows the salyrgan effect, for an incubation time of 60 minutes, as a function of the molar ratio of inhibitor to actin. The polymerizability completely disappeared when this ratio was higher than 4 or 5, in close agreement with the reported number of SH groups in G-actin. (Kominz et al, 1957).

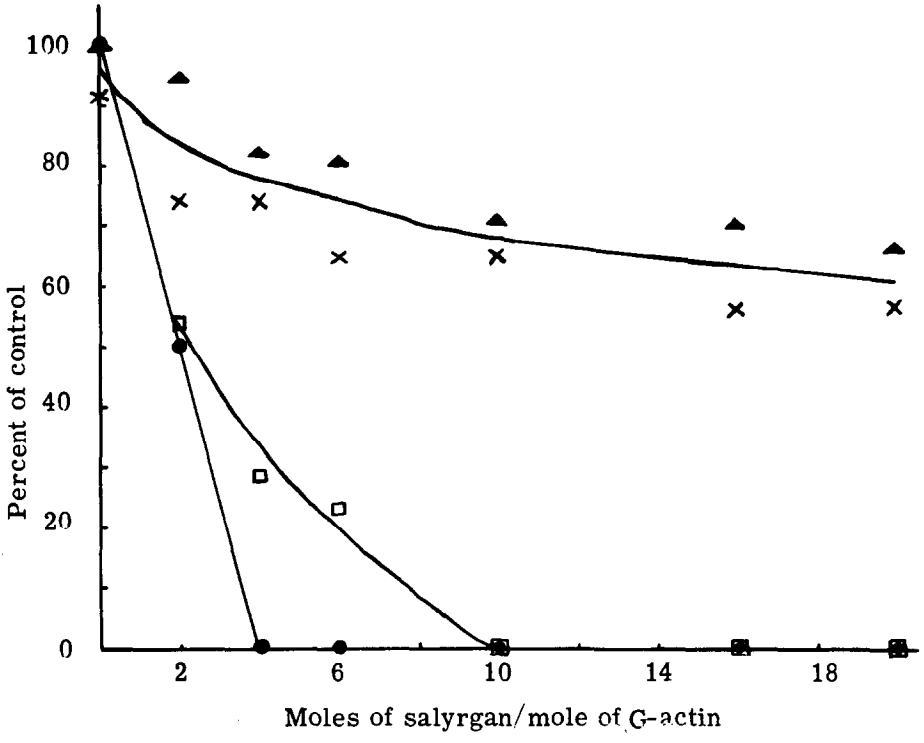

Fig. 2. Comparison of inhibition of actin polymerizability and loss of bound ATP as a function of salyrgan to actin ratio.

G-actin-C^{14}-ATP (Fig. 1), at a concentration of 3.1 mg per ml, was incubated with various amounts of salyrgan at 23° in 2 mM Tris, pH 7.7, for 60 minutes. The polymerizability and bound nucleotide content were measured as described in Fig. 1. ▲——▲: bound nucleotide; ●——●: DRF after polymerization without glutathione; ×——×: DRF after polymerization with glutathione; □——□: DRF after polymerization (without glutathione) of actin incubated with salyrgan and subsequently treated with Dowex-1.

On addition of glutathione there was a recovery of polymerizability, but only to an extent corresponding to the ATP remaining bound. This suggests an irreversible change in actin paralleling the loss of bound ATP. The presence of 1 mM ATP during the incubation with salyrgan had no effect on the loss of polymerizability.

When a sample of G-actin was incubated with salyrgan for 5 minutes and then treated with Dowex the polymerizability, somewhat surprisingly, was partially recovered, even at a ratio of 20 moles of salyrgan per mole of actin. This partial recovery decreased with longer times of incubation; e.g., after 60 minutes (Fig. 2) no recovery was observed with 10 moles of salyrgan per mole of actin. Since salyrgan is readily absorbed by Dowex, these observations open the possibility that, initially, some of the salyrgan inhibition may not be due to a specific interaction with SH groups.

Studies are now in progress to compare the effect of various mercurials. It appears that p-hydroxymercuribenzoate behaves similarly to salyrgan except that the rate of ATP release is about ten times faster. The action of methyl mercury hydroxide is even more rapid, and with this compound it has not yet been possible to separate the effect on polymerizability from that on ATP binding.

The results clearly show that the free SH groups necessary for polymerization of G-actin are distinct from those that may be required for nucleotide binding. At present it is difficult to decide whether the slow release of ATP under the influence of salyrgan is due to a slow reaction with SH groups involved in the binding of ATP, or, more indirectly, to changes produced by the mercurial in the structure of actin.

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