REMOVAL OF THE BOUND CALCIUM OF G-ACTIN BY ETHYLENEDIAMINETETRAACETATE (EDTA)[1]

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The inhibition of the polymerization of actin, accompanied by the release of the bound ATP, by stoichiometric amounts of EDTA was first shown by Martonosi and Gouvea (1961), and independently confirmed by several workers (Strohman (1961), Barany et al. (1961), Grubhofer and Weber (1961), Tonomura and Yoshimura (1961)[3]).

The effect of EDTA may be due to either (a) its binding to actin or (b) the removal of some divalent cation from the protein. The purpose of the present work was to examine these two possibilities without, however, attempting to settle the mechanism of the effect on ATP binding.

To test the first hypothesis, the method of dialysis equilibrium with the use of C^{14}-EDTA was employed. Owing to the impermeability of the Visking cellulose membranes for EDTA in the absence of salts, as was found earlier for ATP (Martonosi et al. 1960), these experiments had to be carried out in 0.1 M KCL. Since the polymerizability of Dowex-1 treated actin in

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3) This paper came to our attention at the time of writing.

the presence of EDTA is lost within 5 minutes at room temperature (Maruyama and Martonosi, 1961), KCL was added 5 minutes after adding EDTA to G-actin that had been previously freed of free ATP by Dowex-1 treatment, thereby avoiding a change in the physical state of actin on adding the salt. As shown in Fig. 1 the equilibrium was reached in about 6 hours without any indication of binding. The time course of the actin-free control agrees quite well with that containing the protein. This agreement would exclude binding at all times up to 24 hours.[4]

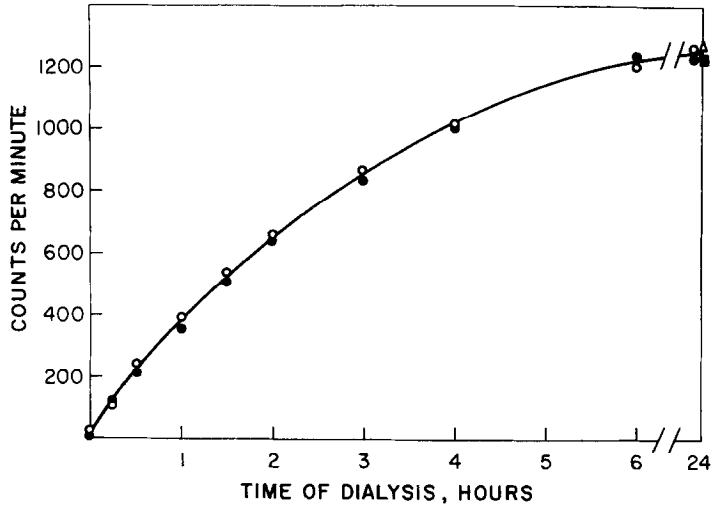

Figure 1. Time course of equilibrium dialysis with C^{14}-EDTA.

Two ml of a solution containing 0.04 mM C^{14}-EDTA with a radioactivity of 7200 c.p.m. per 0.2 ml, 10 mM Tris, pH 8, and 0.033 mM G-actin were dialyzed against 10 ml Tris - 0.1 M KCl, with shaking, at 3°. Radioactivity measurements were made on 0.2 ml aliquots of the dialyzate as described by Drabikowski et al. (1961). In control experiments actin was omitted, but 0.033 mM $CaCl_2$ and ATP were added to the dialysis bag. Key: O, with actin; ⊘, actin-free control; Δ and ■ show the radioactivity inside the bag with and without actin, respectively.

To test the second hypothesis, the Ca content, found by Straub and Feuer (1950) to be 2 moles/mole of actin (MW = 60,000) in crude preparations,

4) At the recent meeting of the Society of General Physiologists (Woods Hole, Mass., September 5-8, 1961), R.C. Strohman presented evidence based on experiments with Sephadex excluding the binding of EDTA to actin

was determined before and after EDTA treatment. As shown in Table I, the Ca content, which was close to 1 mole/mole in our purified actin preparations, was reduced to essentially zero by exposure to EDTA. This finding is in agreement with the results of Tonomura and Yoshimura (1961), but is at variance with those of Chrambach et al. (1961) suggesting no removal of Ca.

TABLE I

Effect of EDTA on the Ca content of G-actin

Sample	Control	EDTA
1	1.5	0.1
2	1.3	0.0
3	1.0	0.2

G-actin, 3-4 mg of protein per ml, was treated with EDTA in a concentration of 5 moles per mole of actin for 5 minutes at 23° C. Then KCl was added to a final concentration of 0.1 M, and the samples were dialyzed over night against 0.1 M KCl - 10 mM Tris Buffer, pH 8.0. The dialyzed sample was deproteinized with 5% perchloric acid, centrifuged, the supernatant neutralized with KOH, and the K-perchlorate removed by centrifugation. Ca determinations in the protein-free supernatant were carried out by the method of Yanagisawa (1959). The values are expressed as moles of Ca per mole of actin.

Another way of showing the removal of bound Ca by EDTA - avoiding, however, the prolonged dialysis involved in the above procedure - is as follows: When Ca^{45} was added to actin treated with EDTA in the cold, actin containing bound Ca^{45}, which retained its polymerizability, was obtained on removing EDTA and free Ca^{45} with Dowex-1 and Dowex-50, respectively.

A second treatment with EDTA led to the immediate loss of bound radioactivity when Dowex-1 was added to remove the Ca-EDTA complex[5] (Fig.2). The fact that bound Ca is removed, in the presence of EDTA, by an anion exchange resin, thus further supports the view that EDTA acts on actin by removing bound Ca, rather than by its binding to the protein.

5) The quantitative removal of Ca^{45}, in the form of the EDTA complex, by Dowex-I in the absence of actin, was shown in the control experiments.

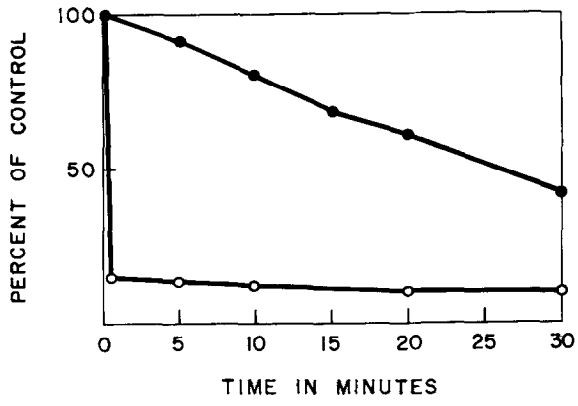

Figure 2. Release of Ca^{45} and loss of polymerizability of G-actin by EDTA

G-actin, 3 - 4 mg of protein per ml in 10 mM Tris buffer, pH 8.0, was first incubated with 10^{-4}M EDTA at 0° C for 2 minutes. Then 10^{-4} M $Ca^{45}Cl_2$ and 10^{-4} M ATP were added; after 5 minutes, excess Dowex-1-Cl to remove Ca-EDTA, EDTA and free ATP; and Dowex-50-W-Na to remove free Ca, were added. After centrifugation the supernatant, containing Ca^{45} -labelled actin, was used for the experiment represented in this figure. The polymerizable actin content as determined by double refraction of flow (DRF) measurements, was 1.35 mg/ml. At zero time 0.13 mM EDTA was added at 0° C and samples were taken at the various times indicated on the abscissa. A one ml aliquot was used to determine the polymerizability by DRF measurements on the addition of 5 mM $MgCl_2$ and 0.05 M KCl, to a total volume of 5 ml. To another one ml aliquot 0.3 ml of Dowex-1-Cl (0.1 meq. per ml) were added to a total volume of 3 ml containing 10 mM Tris, pH 8.0. After shaking for 5 minutes and subsequent centrifugation the radioactivity of an 0.2 ml aliquot of the supernatant was determined (see legend of Fig. 1). Key: ●, polymerizability; O, radioactivity.

It is interesting to note that actin, even after the removal - as the EDTA complex - of its bound Ca loses its polymerizability only relatively slowly at 0° (fig. 2). This fact suggests that whatever the nature of the irreversible change in the actin molecule following EDTA treatment may be, it takes place at a much slower rate than the loss of Ca.

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