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PN 1159

Starch-gel electrophoresis and ultracentrifugation of actin

Prompted by an observation made in checking the homogeneity of G-actin (prepared according to MOMMAERTS[1] from a rabbit-muscle extract according to STRAUB[2]) by means of starch-gel electrophoresis we have performed several experiments, whose results are illustrated by Fig. 1. In starch-gel electrophoresis[3] both in the continuous borate system according to SMITHIES[3] and the discontinuous Tris–borate system according to POULIK[4], the STRAUB extract yields several bands, designated in Diagram 1 by a–d and I–IV*. Upon conversion of G-actin to F-actin by adding KCl to 0.1 M and $MgCl_2$ to 0.001 M concentration to the extract and spinning down the F-actin, the supernatant and a solution of the washed F-actin pellet yielded Diagrams 2A and 2B, respectively. In the supernatant diagram the bands a–d, observed in the extract, are always present; in general the bands I–IV have completely disappeared, only in rare cases a trace of band II was observed. Obviously the bands I–IV in Diagram 1 represent G-actin, while the proteins in bands a–d have no connection with the formation of F-actin. Diagram 2B shows that most of the F-actin is retained in the slot. A minor fraction is present in a diffuse zone, distinctly separated from the fraction in the slot. Upon converting the washed total F-actin pellet to G-actin by adding ATP to 10^{-4} M concentration a diagram is obtained with the bands I–IV only (Diagram 3). This diagram (also in respect of the ratios of the intensities of the stained bands) is not altered by repeatedly converting G-actin to F-actin, and reversely, suggesting that the G-actin components as such are incorporated into the F-actin. Also the diagram of F-actin prepared from purified G-actin is identical with Diagram 2B, and remains so upon repeatedly converting G-actin to F-actin. Re-electrophoresis of the individual isolated G-actin bands I–IV yielded only one band each, located at exactly the same site as before (Diagrams 4 A–D). Hence, if they represent aggregates of a certain unit, their formation does not seem to be reversible. This is also suggested

* Although we always speak of bands I–IV, occasionally 1 or 2 very faint additional bands nearer to the origin could be observed.

by the constancy of the G-actin diagrams upon repeatedly converting G to F. The bands cannot be caused by G-actin molecules bearing different amounts of nucleotides, as the bands I–IV do not disappear upon removing the nucleotides according to the norit method of BÁRÁNY[5]. On the contrary, one or more bands, although very faint,

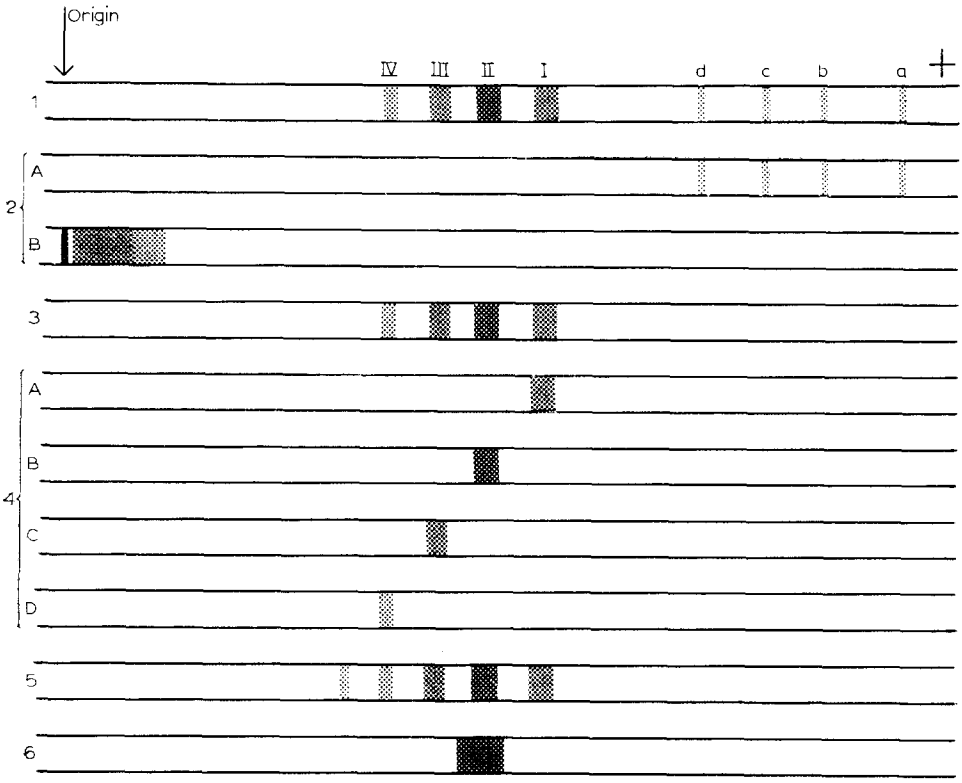

Fig. 1. Starch-gel electrophoresis diagrams. 1, STRAUB extract. 2, Conversion of G-actin to F-actin in STRAUB extracts, followed by ultracentrifugation; A, supernatant; B, sediment (F-actin). 3, G-actin (formed from F-actin). 4A–D, Re-electrophoresis of isolated G-actin fractions. 5, G-actin 4-times treated with norit. 6, Reduced and alkylated G-actin.

nearer to the origin, are then always observed (Diagram 5). Moreover, in agar-gel electrophoresis one band is obtained. Hence the charge of the components observed in starch-gel electrophoresis is the same.

All these observations together suggest that these components are stable polymers. As they may have arisen (either under natural conditions or as artefacts) by the formation of S–S linkages, G-actin in 6 M urea solution was subjected to reduction by mercaptoethanol, followed by alkylation with monoiodoacetate[6]. Only one band was now observed in starch-gel electrophoresis, carried out either in Tris gel prepared in 8 M urea or, after dialysis, in Tris buffer only.

Both the original G-actin and the reduced and alkylated G-actin were studied in the ultracentrifuge. One peak was always observed. In water the s_{20} values found were 2.8 S, (refs. 1 and 7) and 0.9 S, respectively, and in 0.1 M KCl (used for eliminating charge effects) 3.7 S and 2.6 S, respectively. Precautions were taken to avoid the

formation of F-actin in the presence of 0.1 M KCl by adding EDTA to a concentration of 0.005 M to the G-actin[8], from which the free nucleotides had been removed by Dowex-1 X8 (Cl^-)* according to ASAKURA[9]. The peaks of non-reduced G-actin were rather broad and not quite symmetrical, the peaks of reduced G-actin sharp and symmetrical. These results, combined with the result of the starch-gel electrophoresis of reduced G-actin, suggest that from heterogeneous G-actin, consisting of various polymers of S–S-linked units, a homogeneous protein of lower molecular weight is formed by reduction.

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* Starch-gel electrophoresis of G-actin with 0.005 M EDTA and of G-actin with 0.005 M EDTA and 0.1 M KCl showed the same bands as in Diagram 3. No formation of F-actin could be detected.

Note added in proof: G-actin prepared according to the recently described method of LAKI, MARUYAMA AND KOMINZ (*Arch. Biochem. Biophys.*, 98 (1962) 323), and which, according to these authors, should be free of tropomyosin gave a starch-gel diagram identical with Diagram 3 in Fig. 1.

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New data on the link between the polysaccharide prosthetic group and protein in ovalbumin

It has been shown that the polysaccharide prosthetic group in ovalbumin is bound to the peptide chain through an aspartic acid residue[1–3]. There are still no publications describing experimental studies of the nature of this bond.

In order to elucidate this question we isolated from an enzymic hydrolysate of ovalbumin a fragment consisting of both the polysaccharide prosthetic group and aspartic acid[4]. It was found to contain 5 mannose, 3 acetylglucosamine and 1 aspartic acid residues. The nitrogen content (4.39 %) was 0.79 % higher than the theoretical value for such a compound (3.60 %). JOHANSEN *et al.*[3] have also found an excess of nitrogen in glucopeptides isolated from ovalbumin. They note that this "excessive" nitrogen is split off as ammonia under the usual conditions of hydrolysis used for splitting amino acid amides.

Recently we succeeded in isolating from the products of a short-term hydrolysis by 2 *N* H_2SO_4 of the polysaccharide–aspartic acid fragment a still smaller fragment which was free of mannose and contained only aspartic acid and glucosamine in