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ACTIN CO-PURIFIES WITH RNA POLYMERASE II

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SUMMARY: RNA polymerase II, [EC2.7.7.6], from the slime mold *Physarum polycephalum*, purified over 4000-fold can contain a protein with an apparent molecular weight of 46,000. This protein is separated from the putative subunits of RNA polymerase II by polyacrylamide gel electrophoresis under non-denaturing conditions, and by chromatography on phosphocellulose. In this report we identify the protein as actin, and we point out that polypeptides of this apparent molecular weight which have been found associated with RNA polymerase II purified from other sources may also be actin from these organisms.

INTRODUCTION: Highly purified RNA polymerase II from a variety of sources often contains a polypeptide with a molecular weight (M_R) of about 46,000 (1-7). The proteins at this M_R are generally found to be present with an apparent stoichiometry of about 0.5 (1-4, 6, 7). In this report we identify the 46,000 M_R protein, in the case of *P. polycephalum*, as actin using the properties of the actin molecule (8-11).

MATERIALS AND METHODS: Enzyme Purification and Gel Electrophoresis. The conditions used for growing and harvesting microplasmodia of *P. polycephalum* and methods used for the purification and assay of RNA polymerase II from this organism, as well as the methods used for gel electrophoresis have been described in detail (1). The unit of enzyme activity is that amount of enzyme which will catalyze the incorporation of 1 nmole UMP into trichloracetic acid insoluble material in 10 min at 30°.

Electron microscopy. The actin-like protein from the slime mold as well as rabbit skeletal muscle actin were dialyzed against 0.01 M 2-(N-morpholino)-ethanesulfonic acid, 5 mM ATP, 2 mM $CaCl_2$, pH 6.8. Both preparations were then adjusted to a protein concentration of 0.2 mg/ml and to 0.1 M KCl. After 30 min at room temperature, carbon coated grids were floated on a drop of the preparations. The samples were then stained with 1% uranyl acetate (aqueous solution) in 0.1 M KCl, rinsed in H_2O, dried and subsequently examined in a Zeiss Elmiscope EM10.

Fingerprinting. The tryptic peptides of the actin-like protein from slime mold and rabbit skeletal muscle actin were compared by fingerprinting on thin layer cellulose plates. Both proteins were alkylated with iodoacetamide in 8 M urea and digested with trypsin (12). The resulting peptides were separated by electrophoresis in the first dimension and chromatography in the second dimension, and visualized with ninhydrin as previously described (12,13).

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RESULTS AND DISCUSSION: After several purification steps followed by column chromatography on an affinity matrix of Heparin-Sepharose, a highly purified RNA polymerase II preparation was obtained (1). Figure 1 (lane 1) shows this material after polyacrylamide gel electrophoresis in the presence of SDS[1]. A polypeptide with an apparent molecular weight of 46,000 is present. Its apparent stoichiometry, with the putative RNA polymerase II subunit of 135,000 M_R taken as 1.0, was found to be about 0.4 in this preparation as judged by gel scanning. In other preparations it was found that this stoichiometry could be as low as 0.2 and as high as 6.0.

When the material which did not bind to the affinity column was collected and subjected to further analysis, it was found to migrate as a single band in polyacrylamide gels after electrophoresis under non-denaturing conditions. When electrophoresed in the presence of SDS it was also found to migrate as a single band and to co-migrate with rabbit muscle actin. Identification of the protein as actin was carried out using three independent techniques. The fingerprint pattern of the tryptic peptides of the 46,000 M_R protein from the slime mold (Fig. 2a) was very similar to the pattern obtained with rabbit skeletal muscle actin (Fig. 2b) and identical to the patterns obtained previously with cytoplasmic actin purified from plasmodia by conventional methods (11). In the presence of 0.1 M KCl, the actin-like protein (Fig. 2c) was also capable of forming filaments, which in electronmicrographs of negatively stained preparations looked very similar to rabbit muscle F-actin (Fig. 2d). The actin-like protein was bound specifically by antibodies to actin, as demonstrated by indirect immunofluorescence (Kelly and Jockusch, unpublished observations). Within the sensitivity limits of this method, no difference could be detected in cross-reactivity between these proteins with the antibody. The specificity of the reaction was ascertained by incubating the actin-like protein from *P. polycephalum* with a control antibody (affinity column purified anti-tubulin). Non-specific binding of this antibody to the actin-like protein was not observed.

[1] Abbreviations: SDS, sodium dodecyl sulfate.

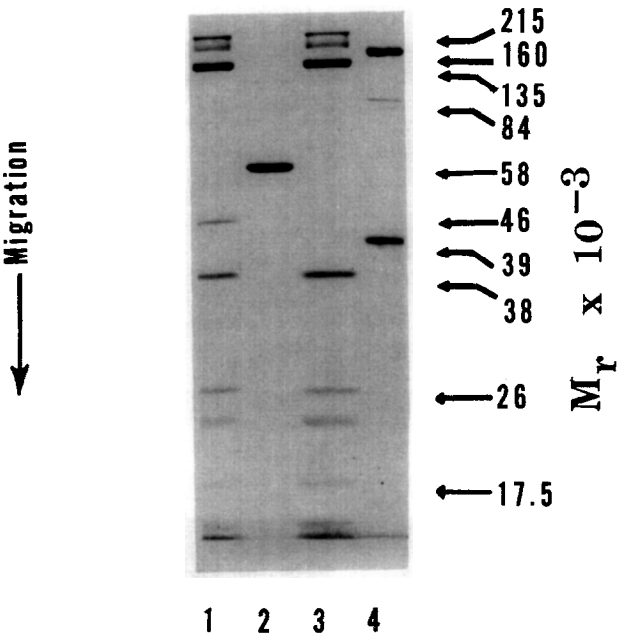

Figure 1: 12.5% polyacrylamide gel electrophoresis of highly purified RNA polymerase II in the presence of SDS. Lane 1: RNA polymerase II after Heparin-Sepharose column chromatography (12 µg). Lane 2: Bovine liver catalase (5 µg). Lane 3: The same RNA polymerase II preparation after phosphocellulose column chromatography (13 µg). Lane 4: *Escherchia coli* RNA polymerase (10 µg).

The protein thus identified as actin was subjected to two-dimensional electrophoresis as previously described (1). In this experiment (Fig. 3a) some of it had aggregated and did not migrate at the same rate as the actin monomer in the first dimension. In the second dimension in the presence of SDS the aggregate was dissociated, and two protein spots appear at 46,000 M_R. The RNA polymerase II preparation shown in Fig. 1 (lane 1) was subjected to electrophoresis in the same two-dimensional system (Fig. 3b). The 46,000 M_R material was separated from the putative RNA polymerase II subunits by electrophoresis under non-denaturing conditions and was found in the center of the pattern at the same location as actin. We conclude that this polypeptide is actin from *P. polycephalum*.

Both Weaver (2) and Smith and Braun (manuscript in preparation) have found that this protein will co-sediment with RNA polymerase II in the presence of 0.5 M KCl, suggesting that it may be bound - perhaps adventitiously - to the RNA

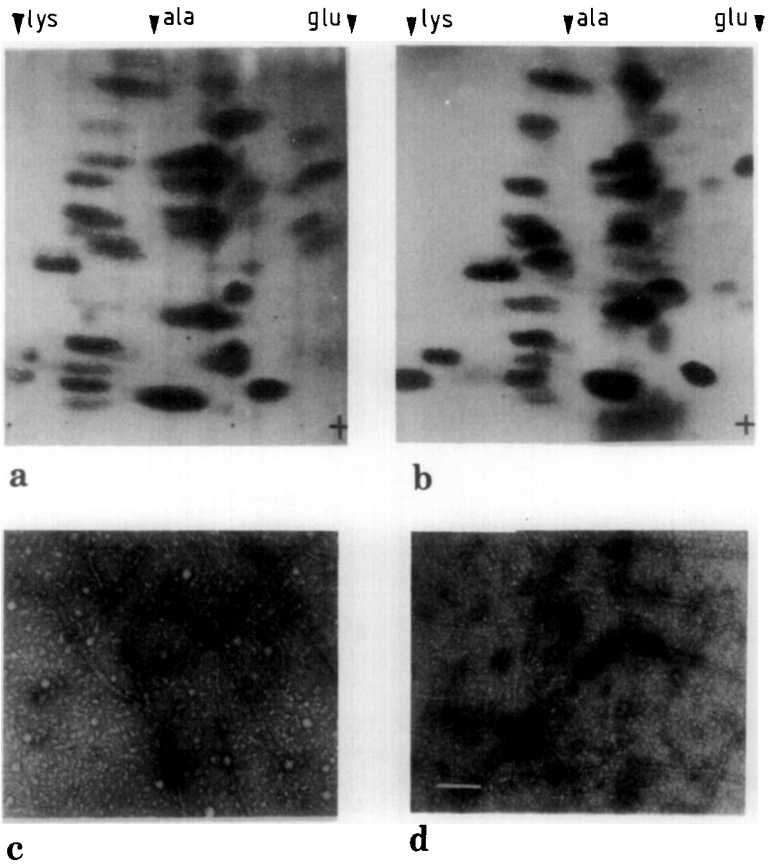

Figure 2: Tryptic fingerprints of rabbit skeletal muscle actin (a) and the actin-like protein from *P. polycephalum* (b) +: start; lys, ala, glu: position of lysine, alanine and glutamic acid used as markers. Uranyl acetate stained filaments formed in solutions of rabbit skeletal muscle G-actin (c) and of the actin-like protein from *P. polycephalum* (d) in the presence of 0.1 M KCl. Bar = 0.1 µm.

polymerase II molecule. It can, however, be removed by chromatography on phospho cellulose (Fig. 1, compare lane 3 with lane 1). After phosphocellulose chromatography the specific activity of the preparation increased from 520 U/mg to 640 U/mg (1). The increase in specific activity correlates well with the removal of the actin as a contaminating protein. However, involvement of actin in eukaryotic RNA synthesis *in vivo* is not precluded by this data.

Preparations of RNA polymerase II from *Saccharomyces cerevisiae* (3-6) contain a polypeptide chain of about 45,000 M_R, which is that reported for actin from

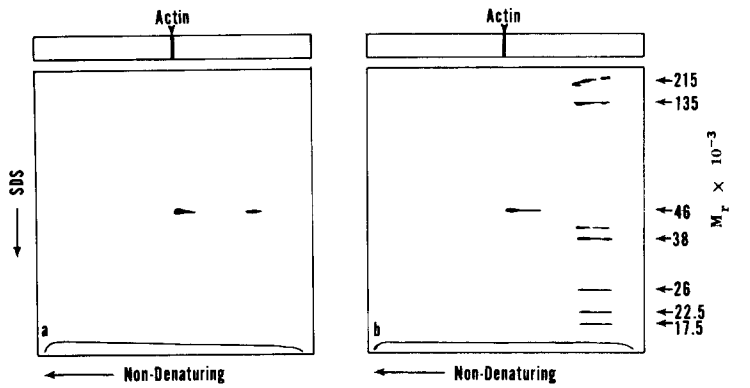

Figure 3: Co-electrophoresis of 46,000 M_R protein in highly purified RNA polymerase II with actin from *P. polycephalum*. Two dimensional electrophoresis was performed as previously described (1). In the first dimension electrophoresis was carried out under non-denaturing conditions in 5% polyacrylamide gel. In the second dimension electrophoresis was carried out in 12.5% polyacrylamide gel in the presence of SDS. a) 12 µg of *P. polycephalum* actin. b) 13 µg of the RNA polymerase II preparation shown in Fig. 1, lane 1. Actin, electrophoresed in a separate experiment under non-denaturing conditions, is shown at the top of each drawing.

this organism (14). Some of these authors (3,4,6) have reported stoichiometries of about 0.5 for this polypeptide. Moreover, both a molecular weight near that of actin and a stoichiometry of about 0.5 have been reported for a polypeptide found in preparations of RNA polymerase II from calf thymus (7). Thus, it does not seem unreasonable to suggest that actin may co-purify with RNA polymerase II from sources other than *P. polycephalum*.

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