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The Polypeptide Subunit Structure of the DNA-dependent RNA Polymerase of *Zea mays* Chloroplasts

(maize nuclear RNA polymerase IIA)

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ABSTRACT **Analysis of the maize chloroplast DNA-dependent RNA polymerase by electrophoresis on polyacrylamide gels that contain sodium dodecyl sulfate shows that the enzyme is multimeric. It contains at least two polypeptides of 180,000 and 140,000 daltons. Polypeptides of lower molecular mass that are associated with the enzyme at several stages of purification may also be subunits of the enzyme.**

Electrophoresis of a mixture of purified maize nuclear DNA-dependent RNA polymerase IIA and chloroplast polymerase on sodium dodecyl sulfate-polyacrylamide gels resolves the 160,000 dalton polypeptide of IIA from the 140,000 dalton polypeptide of the chloroplast enzyme, but does not resolve the 180,000 dalton components found in both enzymes. The polypeptides of less than 40,000 daltons in the highly purified maize nuclear IIA preparations are absent from preparations of purified maize chloroplast RNA polymerase.

DNA-dependent RNA polymerases with distinctively different properties have been isolated from nucleoli, nucleoplasm, mitochondria and chloroplasts of various plants and animals (1). Polypeptide subunit structures of polymerases from each of these sources except chloroplasts have been described (1). In this report we present evidence from sodium dodecyl sulfate ($NaDodSO_4$) polyacrylamide gel electrophoresis that maize chloroplast RNA polymerase is multimeric; it contains polypeptides with molecular masses of 180,000 and 140,000 daltons. A few smaller polypeptides may also be components of the polymerase.

Some properties of maize chloroplast DNA-dependent RNA polymerase have been compared with maize nuclear RNA polymerase IIA (2). The subunit structure of the maize nuclear enzyme IIA has been described (3). Elucidation of the polypeptide subunit structure of the chloroplast polymerase of maize has permitted direct comparisons of the two maize enzymes by $NaDodSO_4$-polyacrylamide gel electrophoresis; they are included in the present report.

Comparative study of the DNA-dependent RNA polymerases of a single eukaryotic cell, as we have undertaken in maize, is necessary to better understand the independence and interdependence of transcription of different genomes at different times during development.

MATERIALS AND METHODS

Isolation of Chloroplasts, Solubilization, and Purification of Chloroplast RNA Polymerase. Maize chloroplasts were isolated and purified, and the RNA polymerase activity assayed and solubilized by methods previously reported (4) except that the solubilization mixture contained 0.5 M KCl and was incubated at 37° for 7 min. The purification schedule of enzyme from 1–1.5 kg of maize leaves is outlined below. Glycerol density gradient, phospho- and DEAE-cellulose purification, modified as noted below for larger scale preparations were also performed as previously reported (4). After each purification step, active enzyme fractions were analyzed by polyacrylamide gel electrophoresis or were pooled for the next purification step.

The purification steps were: 1. *Glycerol density gradient centrifugation* of 12 ml of solubilization mixture containing approximately 100 units of activity was performed by layering 2 ml of mixture on each of six SW 40 tubes. Each tube contained 11.2 ml of a 10–30% linear gradient of glycerol. The gradients were centrifuged at 150,000 × *g* for 20 hr and were fractionated into 1- or 2-ml fractions. 2. *Phosphocellulose chromatography* was performed by applying the Step 1-purified enzyme containing 80–90 units to a 0.6 × 15-cm column and eluting with a 12-ml linear gradient of 0–1.0 M KCl. Fractions of 0.5 ml were collected. 3. Purification of Step 2 enzyme containing 40–50 units of activity by *one* of the following steps: 3a. *DEAE-cellulose chromatography.* 3b. *Rechromatography on phosphocellulose.* 3c. *Chromatography on native DNA-cellulose* prepared by the method of Alberts (5). The Step 2-purified enzyme was applied to a 1-ml column of maize leaf DNA-cellulose. The column was washed with 1.5-column volumes of each of the following: 0.05, 0.1, 0.3, 0.5, 1.0, and 3.0 M KCl. Fractions of 0.5 ml were collected. Enzymatic activity was eluted in the two fractions at 0.3 and 1.0 M KCl.

One *enzyme unit* represents one nmol [^{14}C]AMP incorporated per 20 min at 48°.

Nuclear IIA RNA polymerase was purified by the method of Hardin *et al.* (6).

Polyacrylamide Gel Electrophoresis. Polyacrylamide gel electrophoresis in sodium dodecyl sulfate was performed by the methods of Weber and Osborn (7). Alkylation of enzyme samples and densitometry of the stained gels were performed as reported by Bennett and Bogorad (8).

Electrophoresis in 5% polyacrylamide gels under conditions designed to maintain native protein structure was performed by the methods of Ornstein (9) and Davis (10). Electrophoresis was performed for 1.5 hr at 2–3 mA per gel. Protein was eluted from sections of these gels for enzymatic assay; analysis was done with $NaDodSO_4$-polyacrylamide gel electrophoresis by

Abbreviation: $NaDodSO_4$, sodium dodecyl sulfate.

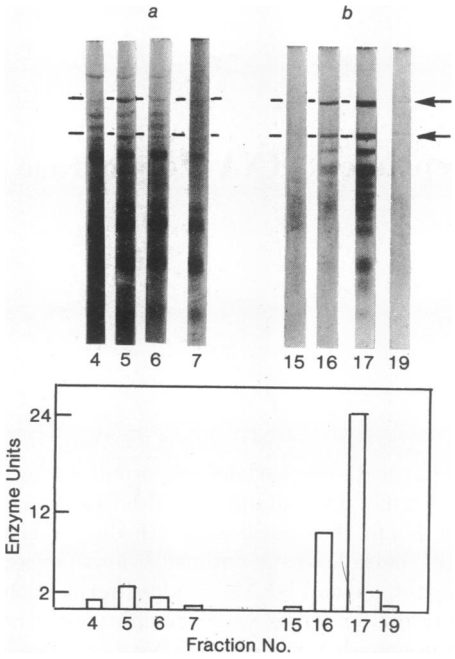

FIG. 1. Sodium dodecyl sulfate polyacrylamide gel patterns and enzymatic activity of fractions taken from across peaks of activity. The 180,000 and 140,000 dalton polypeptides are indicated by *arrows*. (*a*) Step 1, glycerol density gradient-purified enzyme; fractions 4, 5, 6, and 7. Approximately 200 μg of protein was applied to the gel of fraction 5. (*b*) Step 2, phosphocellulose-purified enzyme; fractions 15, 16, 17, and 19 of eluate. Approximately 40 μg of protein was applied to the gel of fraction 17. Protein concentrations were estimated by the method of Lowry *et al.* (20).

placing gel sections into dialysis bags and dialyzing against assay buffers for 18 hr.

Escherichia coli core RNA polymerase and myosin were gifts of David Ratner and Sam Lehrer, respectively.

RESULTS

Photographs of stained $NaDodSO_4$-polyacrylamide gels of maize chloroplast RNA polymerase samples at various steps of purification are presented in Fig. 1. Polypeptides of molecular weight 180,000 ± 10% and 140,000 ± 10% are present at all stages of purification. These polypeptides are among the high molecular mass polypeptides of Step 1-purified enzyme (Fig. 1*a*); they are the only remaining high molecular mass polypeptides after Step 2 purification (Fig. 1*b*); and the only bands seen after Step 3a, DEAE-cellulose purification (Fig. 2*a*). They are also seen in enzyme samples purified through Steps 3b, phosphocellulose rechromatography, and 3c, DNA-cellulose chromatography (Fig. 2*b* and *c*).

Step 1-purified enzyme can be separated from some other proteins by electrophoresis on nondenaturing polyacrylamide gels. Active enzyme eluted from a section of the gel contains both large polypeptides (Fig. 3*a* and *b*).

Regardless of the stage of purification of the enzyme analyzed (Fig. 1), the intensity of staining of the two bands, corresponding to the two polypeptides, is greater the larger the number of units of enzyme applied to the gel. Densitometric tracings of all stained gels indicate that the relative amount of protein in the two bands is roughly equivalent and constant throughout purification.

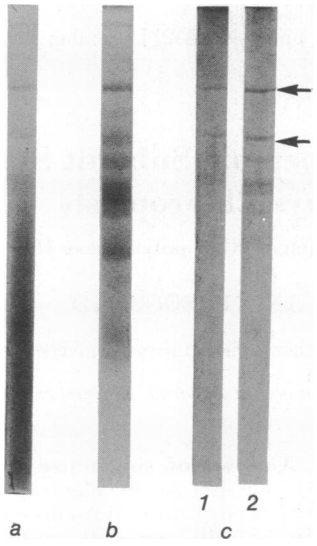

FIG. 2. $NaDodSO_4$-polyacrylamide gel patterns of Step 2 maize chloroplast RNA polymerase further enriched by one of three procedures of Step 3. (*a*) Chromatography on DEAE-cellulose (Step 3a), 1.9 units of activity applied. (*b*) Rechromatography on phosphocellulose (step 3b), 5.8 units. (*c*) Chromatography on maize DNA-cellulose (step 3c) (*1*) 2.6 units of activity eluted at 0.3 M KCl. (*2*) 1.9 units of activity eluted at 1.0 M KCl from the same column.

The 180,000 and 140,000 dalton polypeptides do not appear to represent aggregations of smaller molecular mass polypeptides since the mobilities of the bands are not altered when enzyme samples are alkylated before they are analyzed by $NaDodSO_4$-polyacrylamide gel electrophoresis.

The chloroplast RNA polymerase has a sedimentation constant larger than 18 S, which indicates a molecular mass in excess of 500,000 daltons (4). Such a mass could be accounted for by the presence of more than one of either of the two large subunits in the active aggregate or by the presence of other smaller polypeptides in addition to one each of the 180,000 and 140,000 dalton species. Polypeptides smaller than 140,000 daltons are seen on the gels containing Step 1-purified samples (Fig. 1*a*), but they are reduced in intensity or virtually eliminated at some later stage of purification. Polypeptides of approximately 100,000, 95,000, 85,000, and 40,000 daltons are associated with enzymatic activity in relatively constant ratios at several later steps of purification, but each of these is reduced or eliminated in at least one purification step. No distinct bands of less than 40,000 daltons can be detected, nor are such bands observed when enzyme samples are analyzed by gradient-slab electrophoresis (K. Apel, personal communication).

Comparison of the nuclear IIA and chloroplast RNA polymerase subunit structure

When samples of the maize chloroplast and nuclear IIA RNA polymerases are analyzed on the same $NaDodSO_4$-polyacrylamide gel, the 180,000 dalton polypeptides of the two enzymes are not resolved (Fig. 4), even after extended periods of electrophoresis—conditions under which the β' (165,000 daltons) and β (155,000 daltons) subunits of the *E. coli* core polymerase are well resolved (11). The 140,000 dalton polypeptide of the chloroplast enzyme and the 160,000 dalton polypeptide of the nuclear enzyme are completely resolved

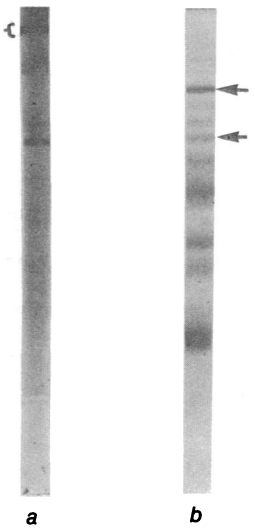

FIG. 3. (*a*) Protein pattern associated with Step 1 maize chloroplast RNA polymerase after electrophoresis of one unit of activity in 5% polyacrylamide under nondenaturing conditions. The gel is stained with Coomassie blue. An enzymatically active sample (0.5 enzyme units) was eluted from sections taken from ten unstained gels each containing the same amount of enzyme and run at the same time as the stained gel shown here. The sections from which enzyme was eluted were slices from the position of the stained band indicated by the bracket. It should be noted that the single band on the nondenaturing gels does not represent a single protein since small amounts of polypeptides of 150,000 and 160,000 daltons, which would be eliminated by phosphocellulose chromatography, are still present in this fraction prepared by electrophoresis under nondenaturing conditions. The 180,000 and 140,000 dalton polypeptides are indicated by *arrows*. (*b*) $NaDodSO_4$-polyacrylamide polypeptide pattern of the enzymatically active fraction eluted as described above.

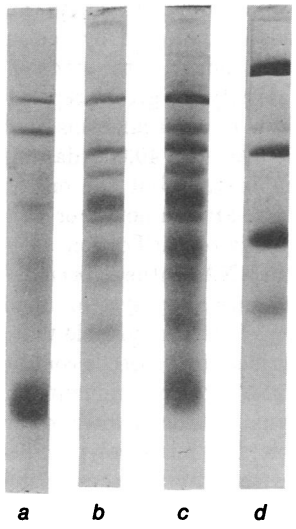

FIG. 4. Sodium dodecyl polyacrylamide gel patterns of samples run in parallel of maize nuclear RNA polymerase IIA and chloroplast RNA polymerase. (*a*) DEAE-cellulose-purified nuclear RNA polymerase IIA. (*b*) Step 2, phosphocellulose-purified chloroplast RNA polymerase. (*c*) Nuclear IIA and chloroplast polymerases. Aliquots of the samples used for gels *a* and *b* were mixed and run on the same gel. (*d*) Reference proteins: myosin, β-galactosidase, bovine serum albumin, and ovalbumin. Samples of maize nuclear IIA RNA polymerase were isolated and purified by James W. Hardin. Relative mobilities of polypeptides associated with the enzymes and reference proteins were the same when reference proteins were mixed with enzyme samples and run as internal standards or externally, as shown here.

and distinct. Polypeptides of less than 40,000 daltons identified in the gel containing the nuclear enzyme are not present in the gel containing the chloroplast enzyme; and conversely, a polypeptide of approximately 40,000 daltons present in the chloroplast enzyme preparation after phosphocellulose chromatography (Step 2) is not present in the nuclear enzyme preparation.

The largest polypeptide of maize nuclear IIA has been reported to be 200,000 daltons (3); and the largest polypeptide of the chloroplast enzyme has been described in preliminary reports as being 220,000 (3), or 200,000 daltons (12). The value reported here for the largest polypeptide of both enzymes is 180,000 ± 10% (Fig. 5). The variation in estimates of molecular mass of this polypeptide may be attributable to differences in the samples of myosin used as reference protein. The myosin samples used for the molecular mass estimations reported earlier have been subsequently found to have a major degradation product with a mobility similar to that of the largest polypeptide of the enzymes. The major polypeptide seen in samples of freshly prepared myosin used as reference protein in the present work has a lower mobility than the large polypeptide of the nuclear IIA and chloroplast enzymes (Fig. 5).

DISCUSSION

In this report we have presented evidence that the maize chloroplast DNA-dependent RNA polymerase is multimeric and contains two polypeptides of relatively high molecular mass. In these respects the chloroplast enzyme is similar to the RNA polymerases of nuclei from several species of plant and animal (1–3, 13–15); but it is different from the small, single polypeptides reported to constitute the RNA polymerase for mitochondria of several species (16–18).

The 180,000 and 140,000 dalton polypeptides are present in chloroplast polymerase samples at all stages of purification, and there is a constant quantitative relationship between the

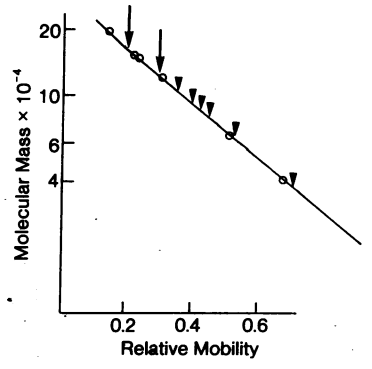

FIG. 5. Standard curve for molecular mass determination of polypeptides associated with maize chloroplast RNA polymerase. *Circles:* Reference proteins and their molecular masses are myosin, 200,000; β-galactosidase, 135,000; β′ and β of *E. coli* core polymerase, 165,000 and 155,000, respectively; bovine serum albumin, 68,000; ovalbumin, 43,000 (21). *Arrows:* large subunits of maize chloroplast RNA polymerase. *Triangles:* other polypeptides associated with the Step 2-purified enzyme seen in Fig. 4*b*.

number of enzyme units applied to the gels and the amount of protein in the bands as indicated by the intensity of band staining. This is independent of the stage of purification and thus of the specific activity of a given preparation.

Other polypeptides of molecular mass of approximately 100,000, 95,000, 85,000, and 40,000 daltons are frequently associated with the enzyme, but one or another of these is sharply reduced in relative amount or cannot be detected after at least one stage of purification. It is possible that a component, active in RNA synthesis *in vivo*—but not essential for activity in an *in vitro* assay system, might be removed at one purification step. It is also possible that bands which become weaker as purification proceeds could be, at early steps of purification, mixtures of contaminants and *bona fide* components of the enzyme. Although positive identification of any of these lower molecular mass polypeptides as part of the enzyme cannot be made by the same criteria as have been applied here for identifying the 180,000 and 140,000 dalton polypeptides as subunits of the enzyme, our data do not rule out the possibility that some or all of these smaller polypeptides are components of the chloroplast RNA polymerase.

Techniques have been developed which allow separation, purification, and reassembly of subunits to form an enzymatically active *E. coli* RNA polymerase (19). This has made it possible to make more positive identification of enzyme subunits, as well as to study their function in RNA synthesis. When such techniques are developed for eukaryotic polymerases, it may be possible to determine whether the low molecular mass components associated with the chloroplast enzyme in almost all, but not every, purified preparation are contaminants or subunits which are lost at certain stages of purification.

Maize nuclear IIA and chloroplast RNA polymerase are similar in that the highest molecular mass component of each is 180,000 daltons; and different in that the second highest molecular mass components are 160,000 and 140,000 daltons, respectively. They are also different in the complement of polypeptides of 40,000 daltons or less. The similarity in the 180,000 dalton polypeptides is suggestive, but hardly conclusive evidence that the enzymes contain a common polypeptide. Further delineation of the structural relationship of the two 180,000 polypeptides awaits application of techniques of analytical protein chemistry. Determination of the functional roles of the subunits of the two enzymes would provide for full characterization and comparison of the enzymes; however, this is not possible at present.

Compartmentalization of RNA polymerases which are different, but nevertheless have subunits which are similar or of common origin might be one basis for interaction and integration of organelle RNA synthesis and function within a eukaryotic cell.

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