M_{412} and M_{405} are modified preparations and caution must be exercised in extrapolating these results to the photoreaction cycle of the intact purple membrane.

References

Becker, R. S., Inuzuka, K., and Balke, D. (1971), *J. Am. Chem. Soc. 93,* 38.
Crouch, R., Purvin, V., Nakanishi, D., and Ebrey, T. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 1538.
Ebrey, T., and Honig, B. (1975), *Q. Rev. Biophys. 8,* 129.
Fransen, M. R., Luyten, W. C. M. M., Van Thuijl, J., Lugtenburg, J., Jansen, P. A. A., van Breugel, P. J. G. M., and Daemen, F. J. M. (1976), *Nature (London) 260,* 726.
Honig, B., and Ebrey, T. (1974), *Annu. Rev. Biophys. Bioeng. 3,* 151.
Jan, L.-Y. (1975), *Vision Res. 15,* 1081.
Lozier, R. H., Bogomolni, R. A., and Stoeckenius, W. (1975), *Biophys. J. 15,* 955.
Lozier, R. H., and Niederberger, W. (1977), *Fed. Proc., Fed. Am. Soc. Exp. Biol.* (in press).
Oesterhelt, D., and Hess, B. (1973), *Eur. J. Biochem. 37,* 316.
Oesterhelt, D., Meentzen, M., and Schuhmann, L. (1973), *Eur. J. Biochem. 40,* 453.
Oesterhelt, D., and Stoeckenius, W. (1974), *Methods Enzymol. 31,* 667.
Pilkiewicz, F. G., Pettei, M. J., Yudd, A. P., and Nakanishi, K. (1977), *Exp. Eye Res.* (in press).
Rosenfeld, T., Honig, B., Ottolenghi, M., Hurley, J., and Ebrey, T. (1977), *Pure Appl. Chem.* (in press).
Stoeckenius, W., and Rowen, R. (1967), *J. Cell Biol. 34,* 365.
Wald, G. (1968), *Science 162,* 230.
Warshel, A. (1976), *Nature (London) 260,* 679.

Studies on the Subunit Structure of Wheat Germ Ribonucleic Acid Polymerase II†

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ABSTRACT: We have previously presented a rapid, high yield method for the large scale purification of homogeneous RNA polymerase II from wheat germ (Jendrisak, J. J., and Burgess, R. R. (1975), *Biochemistry 14,* 4639), and we now report a detailed study of its subunit structure. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate indicates that polypeptides with molecular weights of 220 000, 140 000, 40 000, 27 000, 25 000, 21 000, 20 000, 17 800, 17 000, 16 500, 16 000, and approximately 14 000 are associated with the enzyme. Two-dimensional polyacrylamide gel electrophoresis, by which the subunits were separated in the first dimension in the presence of 8 M urea at pH 8.7 and in the second dimension in the presence of 0.1% sodium dodecyl sulfate, indicates that the 40 000 molecular weight component is composed of two nearly identical polypeptides and that the low molecular weight components (≤40 000) are acidic proteins except for the 25 000 molecular weight polypeptide.

In every eukaryotic system examined, multiple nuclear RNA polymerases have been detected, and due to recent advances in methodology for the purification of these enzymes (Schwartz and Roeder, 1975; Greenleaf and Bautz, 1975; Buhler et al., 1974; Valenzuela et al., 1976) there has been rapid progress in elucidation of the complex physical structure in those instances where sufficient amounts of homogeneous protein have been available for analysis. We have recently described a method for the rapid, large-scale purification of milligram quantities of homogeneous RNA polymerase II from wheat germ (Jendrisak and Burgess, 1975). This enzyme is inhibited by low concentrations of α-amanitin (<0.1 μg/mL) and is probably the enzyme involved in the synthesis of heterogeneous nuclear RNA. The ease of purification and availability of large quantities of wheat germ RNA polymerase II make it attractive for further studies on the physical properties of eukaryotic RNA polymerase and for possible enzyme reconstitution from isolated subunits. We have examined in detail the polypeptide subunit composition of this enzyme in order to establish criteria for enzyme purity and as prerequisite information for further studies on subunit function and reconstitution.

Experimental Procedures

Materials. The following electrophoresis reagents were purchased from Bio-Rad: acrylamide, methylenebisacrylamide, ammonium persulfate, tetraethylmethylenediamine and sodium dodecyl sulfate. Tris[1] base and glycine were purchased from Sigma. Coomassie brilliant blue and urea (Ultrapure) were purchased from Schwarz/Mann. The following protein molecular weight markers were obtained from Worthington: yeast pyruvate kinase, ovalbumin, bovine pancreatic chymotrypsinogen, and *Escherichia coli* β-galactosidase. Bovine serum albumin was purchased from Miles; hemoglobin and

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[1] Abbreviations used: Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

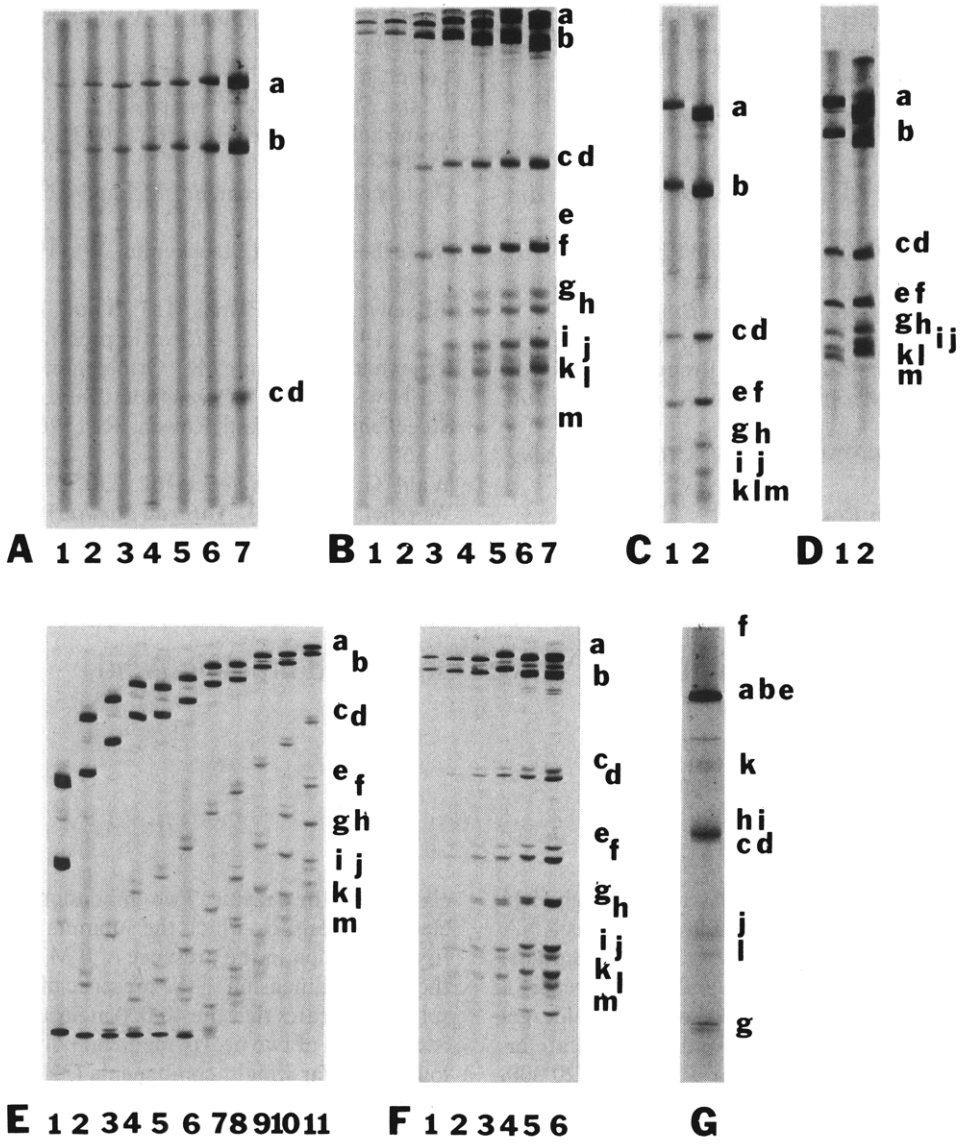

FIGURE 1: Polyacrylamide gel electrophoresis patterns of wheat germ RNA polymerase II under denaturing conditions. The enzyme was purified as described previously (Jendrisak and Burgess, 1975). All bands migrated toward the anode, from top to bottom. (A–D) Sodium dodecyl sulfate–polyacrylamide gels run in a sodium phosphate (pH 7.0) buffer system. (A) Five percent polyacrylamide gels, 1–7: 0.8, 1.6, 2.4, 3.2, 4.0, 8.0, and 20 μg of protein. (B) Ten percent polyacrylamide gels, 1–7: 2, 4, 8, 16, 24, 32, and 40 μg of protein. (C) Gels composed of 5% polyacrylamide in the top half and 10% polyacrylamide in the bottom half: 1, 8 μg of protein; 2, 20 μg of protein. (D) Five to twenty percent polyacrylamide linear gradient gels: 1, 20 μg of protein; 2, 40 μg of protein. (E and F) Sodium dodecyl sulfate–polyacrylamide gels run in a Tris–glycine, discontinuous (stacking) buffer system: (E) 1–11, 15 μg of protein on gels containing 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15% polyacrylamide; (F) 1–6, 2.5, 5, 10, 15, 25, and 35 μg of protein on 12.5% polyacrylamide gels. (G) Urea gel, 8 M, run in a Tris-glycine discontinuous buffer system. Fifteen micrograms of protein was applied to a 7.5% polyacrylamide gel. Additional details on running, staining, etc. can be found in the text. Polypeptides are indicated by letters which correspond to the same polypeptides in Figures 2 and 3 and in Table I.

bovine β-lactoglobulin were purchased from Schwarz/Mann; rabbit skeletal muscle phosphorylase *a* was purchased from Boehringer. Horse heart cytochrome *c* was purchased from Calbiochem and rabbit skeletal muscle myosin was the generous gift of Dr. Fay Yang.

DNA-Dependent RNA Polymerase II. Wheat germ RNA polymerase II was purified essentially as described by Jendrisak and Burgess (1975). The storage conditions have been modified, however, and are reported here. The purified polymerase eluted from the phosphocellulose column was dialyzed for 12 h each against two changes (20 volumes) of a storage buffer containing 0.05 M Tris-HCl (pH 7.9 at 25 °C), 50% (v/v) glycerol, 1 mM EDTA, 1 mM dithiothreitol, and 0.1 M NaCl. This resulted in a twofold concentration of the enzyme. After determining the protein concentration by spectral analysis and the $E_{280}^{1\%} = 7.7$ (Jendrisak et al., 1976), the enzyme was aliquoted into capped plastic ampules and stored frozen at −70 °C where it is indefinitely stable. The aliquot in use is stored at −20 °C unfrozen.

Polyacrylamide Gel Electrophoresis. This was performed in 0.5-cm diameter glass cylinders as described by Burgess and Jendrisak (1975). Two gel systems containing 0.1% sodium dodecyl sulfate were used. A continuous (nonstacking) $NaPO_4$ (pH 7.0) buffer system was run by the general method of Shapiro et al. (1967). A discontinuous (stacking) Tris–glycine (pH 8.7) buffer system (Ornstein, 1964; Davis, 1964) was run by the general method of Laemmli (1970). Gels containing 8 M urea in the discontinuous Tris–glycine system were run according to Jovin et al. (1964). All gels contained acrylamide and bisacrylamide in a ratio of 37.5 to 1.

TABLE I: Polypeptide Composition of Wheat Germ RNA Polymerase II.

Polypeptide	Mol Wt[a]	Molar Ratio[b]
a	220 000	0.9
b	140 000	1.0
c	40 000	0.4
d	40 000	0.6
e	27 000	0.4
f	25 000	1.4
g	21 000	1.0
h	20 000	1.0
i	17 800	1.3
j	17 000	0.8
k	16 300	1.4
l	16 000	1.0
m	(14 000)[c]	(1)[c]

[a] Molecular weights of polypeptides were determined by simultaneous electrophoresis of RNA polymerase with protein standards in sodium phosphate buffered sodium dodecyl sulfate gels (see Figure 3). [b] Molar ratios were determined by electrophoresis in Tris–glycine-buffered sodium dodecyl sulfate gels which were subsequently stained with Coomassie blue and quantitated by densitometry at 550 nm (see Figures 4 and 5). Molar ratios were normalized to polypeptide b. [c] These values are uncertain due to limitations in determining molecular weights for very small polypeptides by the polyacrylamide gel systems used (Dunker and Rueckert, 1969).

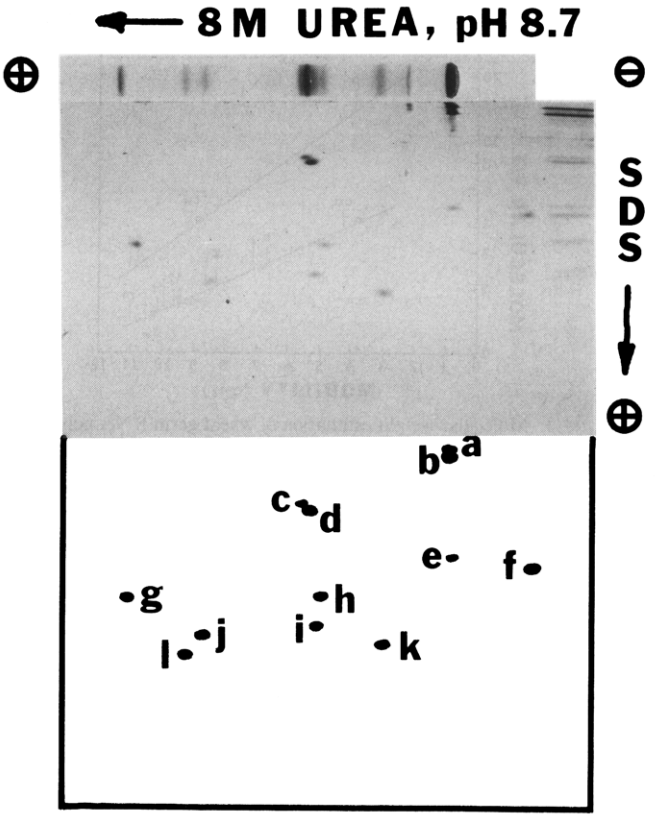

FIGURE 2: Two-dimensional polyacrylamide gel electrophoresis of wheat germ RNA polymerase II. The first dimension (horizontal) was an 8 M urea gel run in a Tris–glycine-buffered system. The gel was 6-cm long and contained 7.5% acrylamide, and the marker dye was run to within 1 cm of the bottom. After electrophoresis, the gel was removed and incubated in sample buffer for electrophoresis in the second dimension which consisted of a 15% polyacrylamide slab gel run in a sodium dodecyl sulfate–Tris–glycine system. Twenty micrograms of protein was subjected to electrophoresis. Additional details are given in the text. Directions of migration are indicated by the arrows. Separate one-dimensional gels in 8 M urea and in sodium dodecyl sulfate are shown on the top and to the right of the slab gel, respectively. In the schematic drawing, polypeptides are indicated by letters which correspond to the same polypeptides in Figures 1 and 3 and Table I.

Two-dimensional gels were run as follows: The first dimension consisted of the 8 M urea, pH 8.7 gels (described above) run in 0.5-cm diameter tube with a length of 6 cm. The marker dye was run within 1 cm of the bottom of the gel and after electrophoresis the gel was immediately equilibrated by soaking in 20 mL of the sodium dodecyl sulfate sample buffer of Laemmli (1970) for 30 min at 37 °C. The gel was placed on top of a 10.0 × 14.0 × 0.3 cm slab gel containing 15% acrylamide and the discontinuous sodium dodecyl sulfate–Tris–glycine gel system described by Laemmli (1970). The gel was formed in an apparatus from Hoefer Instruments, Los Angeles, as detailed by O'Farrell (1975).

Gels were stained with Coomassie brilliant blue as described by Burgess and Jendrisak (1975) ensuring saturation of the gel with stain and guarding against overdestaining which results in perferential loss of dye bound by the lower molecular weight polypeptides (Burgess, 1976).

Molecular weights of polypeptides were estimated by simultaneous electrophoresis of RNA polymerase with molecular weight standards in polyacrylamide gels containing 0.1% sodium dodecyl sulfate by the general method of Weber and Osborn (1969). Molar ratios were determined by densitometric scanning of stained gels at 550 nm in a Beckman Acta III recording spectrophotometer equipped with a gel scanning attachment at a slit width of 0.1 mm and a scan rate of 1.6 cm/min (Burgess and Jendrisak, 1975).

Results

Several polyacrylamide gel electrophoresis systems run under denaturating conditions were utilized in order to examine the putative polypeptide subunits of wheat germ RNA polymerase II (Figure 1). Also variations in the amounts of protein applied to the gel and variations in the percent acrylamide used in the gel were made in order to optimize detection and separation of all of the polypeptide components. Finally two-dimensional polyacrylamide gel analysis was performed in order to resolve all of the polypeptide components (Figure 2).

Subunit Molecular Weights. All of the data indicate that wheat germ RNA polymerase II, like other eukaryotic RNA polymerases, displays a very complex subunit structure composed of 2 high molecular weight polypeptides (greater than 140 000) and 11 smaller polypeptides of molecular weight 40 000 or less. The molar ratios of some of these polypeptides are near unity; however, several polypeptides appear to be present in lower or higher than stoichiometric amounts. A summary of the subunit analysis of wheat germ RNA polymerase II appears in Table I.

Molecular weights of polypeptides were determined in 5 and 10% acrylamide–0.1% sodium dodecyl sulfate gels run in the continuous $NaPO_4$ (pH 7.0) buffer system since it has been well-documented that polypeptides reliably migrate according to their molecular weights (Weber and Osborn, 1969; Dunker and Rueckert, 1969). In gels containing the discontinuous Tris–glycine (pH 8.7), stacking buffer system, other factors besides molecular weight may influence migration (Swaney et al., 1974). The molecular weight determinations appear in Figure 3. Markers were run simultaneously with RNA polymerase in the same gel. Using molecular weight markers of

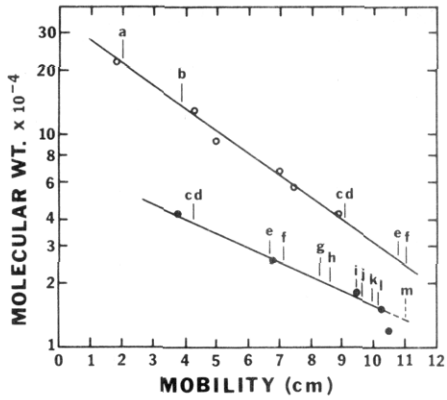

FIGURE 3: Molecular weight estimation of wheat germ RNA polymerase II polypeptides sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The positions of the polypeptides are indicated by vertical lines and the letters correspond to the same polypeptides in Figures 1 and 2. The standard curves were made from the migration of proteins of known molecular weights (see Results). Gels contained 5% (○) or 10% (●) acrylamide in a $NaPO_4$ (pH 7.0) continuous buffer system.

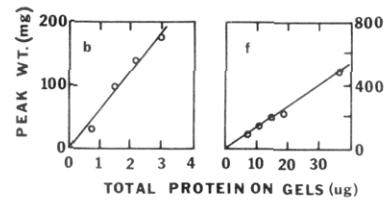

FIGURE 5: Molar ratio estimation of the subunits of wheat germ RNA polymerase II. Shown here is the type of molar ratio analysis carried out using polypeptides b and f as examples. Graded levels of protein were applied to 5 and 12.5% polyacrylamide gels containing 0.1% sodium dodecyl sulfate and using a Tris–glycine (pH 8.7) discontinuous (stacking) buffer system (for example, see Figure 1F; gels 1–6). Five percent polyacrylamide gels are not shown. Gels were stained with Coomassie blue and then destained until the background absorbance at 550 nm was approximately 0.1. Gels were scanned using a Beckman Acta III recording spectrophotometer at a slit width of 0.1 mm and a scan rate of 1.6 cm/min. The lines represent the relationship of dye bound (the area under the scan peaks was determined by cutting out the peaks and weighing them) to the amount of protein applied to the gel. Molar ratios were determined from the slopes of the best fitting lines through the points, by dividing these slopes by the molecular weights for the corresponding polypeptides determined from Figure 3. Ratios were then normalized to polypeptide b.

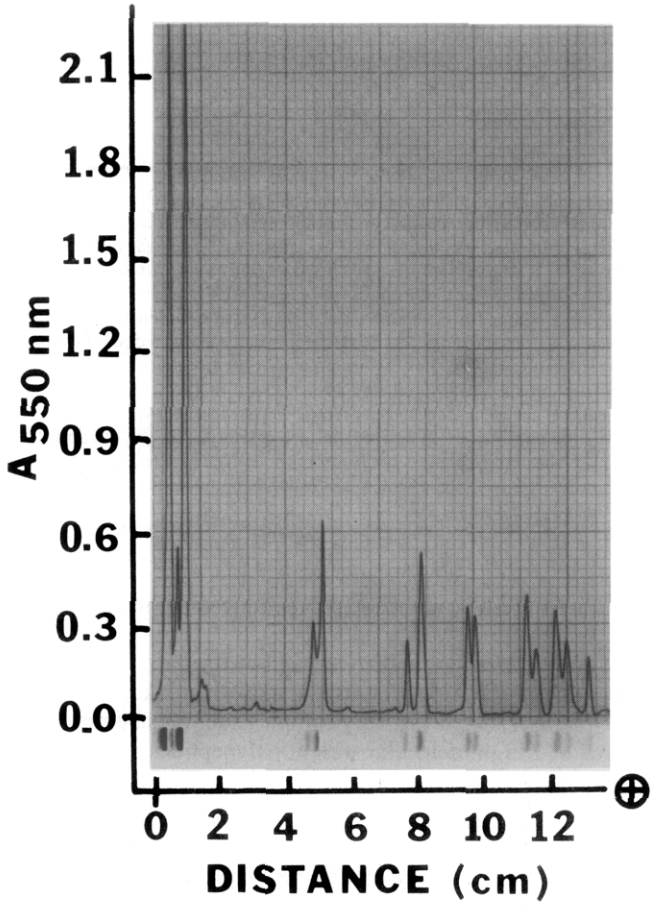

FIGURE 4: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis pattern and corresponding densitometer tracing of wheat germ RNA polymerase II. Protein (15 μg) was applied to a 12.5% acrylamide gel containing 0.1% sodium dodecyl sulfate and using a Tris–glycine buffer system. The gel was stained with Coomassie blue and scanned as described in the text. The origin is at the left and subunits migrated toward the right in order of decreasing molecular weight.

myosin 220 000 (Woods et al., 1963; Gershman and Dreizen, 1969), β-galactosidase 130 000 (Ullmann et al., 1968), phosphorylase *a* 94 000 (Ullmann et al., 1968), bovine serum albumin 68 000 (Tanford et al., 1967), pyruvate kinase 57 000 (Steinmetz and Deal, 1966), ovalbumin 43 000 (Castellino and Barker, 1968), chymotrypsinogen 25 700 (Weber and Osborn, 1969), β-lactoglobulin 18 400 (Tanford et al., 1967), hemoglobin 15 500 (Weber and Osborn, 1969), and cytochrome *c* 12 000 (Weber and Osborn, 1969), the molecular weights of the RNA polymerase II subunits are: 220 000, 140 000, 40 000, 27 000, 25 000, 21 000, 20 000, 17 800, 17 000, 16 500, 16 000, and one component less than 14 000. There are uncertainties in defining accurate molecular weights for the two high molecular weight subunits due to the lack of reliable high molecular weight markers. Also the band at less than 14 000 (polypeptide m) is of uncertain molecular weight since the standard curve deviates from linearity in this region (Figure 3 and Dunker and Rueckert, 1969).

Subunit Molar Ratios. Molar ratios of the polypeptides (Table I) were determined by densitometric scanning at 550 nm of gels containing sodium dodecyl sulfate (Laemmli, 1970) run in the high resolution Tris–glycine discontinuous (stacking) buffer system (Ornstein, 1964; Davis, 1964). Various amounts of protein were run on 5% (data not shown) and 12.5% (Figure 1F) acrylamide gels in order to ensure a linear relationship between the absorbance areas under the scan peaks and the amount of protein applied to the gel. The type of analysis carried out is illustrated in Figure 5 where the molar ratios of polypeptide f and polypeptide b were determined from the slopes of the lines and the molecular weights assigned from Figure 3.

Polypeptide Subunit Patterns on Various Polyacrylamide Gel Electrophoresis Systems. Several observations were made concerning polyacrylamide gel electrophoresis of RNA polymerase under denaturing conditions. When the enzyme is subjected to electrophoresis in the presence of sodium dodecyl sulfate and in the continuous (nonstacking) pH 7.0 phosphate buffer system (Figures 1A–D), one polypeptide is observed with a molecular weight of approximately 40 000 (labeled cd) on gels of various acrylamide concentrations and with various amounts of protein loaded. This band is clearly resolved into two polypeptides on sodium dodecyl sulfate–Tris–glycine (pH 8.7) polyacylamide gels of every gel concentration tried between 7 and 15% acrylamide (Figure 1E; gels 2–11; bands c and d). Interestingly these two polypeptides migrate similarly on the 8 M urea, pH 8.7 gels (Figure 2, spots c and d) which

separate polypeptides on the basis of charge as well as molecular weight. The second example concerns the two polypeptides with molecular weights of 21 000 and 20 000 (polypeptides g and h, Table I). On 15% acrylamide–sodium dodecyl sulfate stacking gels, only one component is seen with a molecular weight of approximately 20 000 (Figure 1E; gel 16, band gh). However, on gels of lower acrylamide concentration, the two polypeptides are resolved (Figure 1E; gels 5–10). These two polypeptides (bands g and h) are resolved on 10% acrylamide–sodium dodecyl sulfate gels in a continuous $NaPO_4$ (pH 7.0) buffer system (Figure 1B, gels 1–7) but not on the gels composed of the top half of 5% acrylamide and the lower half 10% acrylamide (Figure 1C) or on gels containing a linear gradient of 5–15% acrylamide (Figure 1D). Resolution of other low molecular weight polypeptides is lost on these latter two gel systems as well. The polypeptides at 21 000 (g) and 20 000 (h) are resolved very well on the 8 M urea gel system (Figure 1G). These polypeptides were identified by their molecular weights from the two-dimensional gel on Figure 2. Polypeptide g migrates with the marker dye, whereas polypeptide h migrates one third as fast, indicating large charge differences in the two polypeptides.

The two high molecular weight components do not enter the separating gel of the 8 M urea, pH 8.7 system and the 27 000 molecular weight polypeptide is present at the gel interface. It is possible that this polypeptide is still bound to one or both of the high molecular weight polypeptides in the presence of 8 M urea. The 25 000 molecular weight polypeptide remains at the top of the stacking gel, indicating that it is unchanged at pH 8.7 and is therefore a basic polypeptide.

In summary, one set of gel conditions (acrylamide concentration, discontinuous vs. continuous buffer systems, or one amount of protein) may give incomplete resolution and detection of all polypeptides present in the enzyme.

Discussion

The data presented here demonstrate that wheat germ RNA polymerase II has a complex quaternary structure which is similar to that of analogous enzymes isolated from yeast and animal sources. Two high molecular weight polypeptides (220 000 and 140 000) and 11 polypeptides with molecular weights less than 41 000 are associated with the purified enzyme (Jendrisak and Burgess, 1975). In comparison, RNA polymerases II from yeast (Buhler et al., 1976) and mouse plasmacytoma (Schwartz and Roeder, 1975) are also composed of two high molecular weight components (greater than 140 000) and seven or eight polypeptides with molecular weights less than 45 000 (Buhler et al., 1976; Schwartz and Roeder, 1975). The 14 500 subunit of some yeast RNA polymerase II preparations can be resolved into two or three components upon isoelectric focusing in the presence of 6 M urea (Buhler et al., 1976). Nevertheless one of the components is always found in every preparation of the purified enzyme. As interesting similarity of the yeast and wheat germ RNA polymerase II concerns the 27 000 yeast RNA polymerase subunit and the 25 000 wheat germ RNA polymerase II subunit. Buhler et al. (1976) have assigned an isoelectric point of 9.15 for the 27 000 polypeptide upon isoelectric focusing under denaturing conditions and it is the only basic low molecular weight polypeptide in yeast RNA polymerase II. The 25 000 subunit of wheat germ RNA polymerase II failed to migrate into pH 8.7, 8 M urea gels, indicating that it is uncharged at this pH and hence is also a basic polypeptide. These two polypeptides may be analogous in function.

Despite precautions taken in staining and destaining of gels prior to densitometric analysis, several of these polypeptides exhibited molar ratios higher or lower than stoichiometric amounts. For example, there are two polypeptides with molecular weight of 40 000 with molar ratios of 0.4 and 0.6. The fact that their sum is 1.0 may indicate that one of the polypeptides may be derived from the other by modification (adenylation, phosphorylation, glycosylation, etc.) which causes slight alterations in mobility on stacking sodium dodecyl sulfate gels. That the two polypeptides migrate similarly on both 8 M urea pH 8.7 gels (Figure 2) and on isoelectric focusing gels (data not shown) in 8 M urea is consistent with this possibility.

Several other factors peculiar to the use of wheat germ for the purification of RNA polymerase and relating to the complex polypeptide patterns and molar ratios should be discussed. The wheat germ used in these studies was derived from bread wheat varieties (*Triticum aestivum*) which are allohexaploid in genotype. There may be several nonidentical but homologous polypeptides from six possible alleles which compete for assembly into functional RNA polymerase molecules. However, the subunit structure of wheat germ RNA polymerase II based on these studies does not appear to be substantially more complex than RNA polymerase II isolated from diploid yeast cells. In addition, wheat germ is composed of several anatomically and physiologically distinct cell types since it is an embryo and composed of several tissues. If there are tissue specific RNA polymerase subunits, a mixture of enzymes has been isolated resulting in the complex subunit structure with many polypeptides of apparent nonstoichiometric ratios.

However, no evidence has been seen of fractionation of wheat germ RNA polymerase II into several forms which might result from the hexaploid and embryonic nature of wheat germ. All of the polypeptides mentioned in Table I appear to be polymerase subunits since they are all found associated with the enzyme activity when purified RNA polymerase II is chromatographed in Bio-Gel A-1.5m (Jendrisak and Burgess, 1975) which should remove weakly adhering low molecular weight contaminants. Also, the polypeptide molar ratios in each column fraction are constant after gel filtration and other chromatographic procedures. In addition, one major band was observed after gel electrophoresis of the purified enzyme under nondenaturing conditions (Jendrisak et al., 1976). Finally, the polypeptide pattern is strikingly similar to the reported subunit structure of other eukaryotic RNA polymerases.

The complexity in subunit structure of eukaryotic RNA polymerases requires that sufficient amounts of highly purified enzyme be available for subunit structural analysis. For example, as shown in Figure 1F, more than 5 μg of enzyme must be applied to the gel to even begin to detect the majority of the polypeptide subunits present. Also these studies demonstrate that a variety of gel electrophoresis conditions must be utilized in order to resolve and detect all of the polypeptides associated with the enzyme. The availability of large amounts of highly purified wheat germ RNA polymerase II and information on its putative subunit structure make it a useful enzyme for possible reconstitution studies. It is only by this technique that we will know that a particular polypeptide is involved in polymerase activity and be able to determine its function. Knowledge of the physical properties and subunit structures of a variety of eukaryotic RNA polymerases isolated from different cell types, tissues, etc. when coupled with studies of their enzymatic or template properties may provide insight into understanding the complex subunit structures of eukaroytic RNA polymerases.

References

Buhler, J.-M., Iborra, F., Sentenac, A., and Fromageot, P. (1976), *J. Biol. Chem. 251,* 1712.
Buhler, J.-M., Sentenac, A., and Fromageot, P. (1974), *J. Biol. Chem. 249,* 5963.
Burgess, R. R. (1976), in RNA Polymerase, Losick, R., and Chamberlin, M., Ed., Cold Spring Harbor, N.Y., Cold Spring Harbor Press, pp 69–100.
Burgess, R. R., and Jendrisak, J. J. (1975), *Biochemistry 14,* 4634.
Castellino, F. J., and Barker, R. (1968), *Biochemistry 7,* 2207.
Davis, B. J. (1964), *Ann. N.Y. Acad. Sci. 121,* 404.
Dunker, A. K., and Rueckert, R. R. (1969), *J. Biol. Chem. 244,* 5074.
Gershman, L. C., and Dreizen, P. (1969), *Biophys. J. 9,* A235.
Greenleaf, A. L., and Bautz, E. K. F. (1975), *Eur. J. Biochem. 60,* 169.
Jendrisak, J. J., and Burgess, R. R. (1975), *Biochemistry 14,* 4639.
Jendrisak, J. J., Petranyi, P. W., and Burgess, R. R. (1976), in RNA Polymerase, Losick, R., and Chamberlin, M., Ed., Cold Spring Harbor, N.Y., Cold Spring Harbor Press, pp 779–791.
Jovin, T. A., Chrambach, A., and Naughton, M. A. (1964), *Anal. Biochem. 9,* 351.
Laemmli, U. K. (1970), *Nature (London) 227,* 680.
O'Farrell, P. O. (1975), *J. Biol. Chem. 250,* 4007.
Ornstein, L. (1964), *Ann. N.Y. Acad. Sci. 121,* 321.
Schwartz, L. B., and Roeder, R. G. (1975), *J. Biol. Chem. 250,* 3221.
Shapiro, A. L., Vinuela, E., and Maizel, J. V. (1967), *Biochem. Biophys. Res. Commun. 28,* 815.
Steinmetz, M. A., and Deal, W. C., Jr. (1966), *Biochemistry 5,* 1399.
Swaney, J. B., Van de Woulde, G. F., and Bachrach, H. L. (1974), *Anal. Biochem. 58,* 337.
Tanford, C., Kawahara, K., and Lapanji, S. (1967), *J. Am. Chem. Soc. 89,* 729.
Ullmann, A., Goldberg, M. E., Perrin, D., and Monod, J. (1968), *Biochemistry 7,* 261.
Valenzuela, P., Weinberg, F., Bell, G., and Rutter, W. J. (1976), *J. Biol. Chem. 251,* 1464.
Weber, K., and Osborn, M. (1969), *J. Biol. Chem. 244,* 4406.
Woods, E. F., Himmelfarb, S., and Harrington, W. F. (1963), *J. Biol. Chem. 238,* 2374.

Estrogen Photoaffinity Labels. 1. Chemical and Radiochemical Synthesis of Hexestrol Diazoketone and Azide Derivatives; Photochemical Studies in Solution†

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ABSTRACT: Two photosensitive estrogen derivatives, hexestrol diazoketopropyl ether (**5**) and hexestrol azide (**8a**), have been synthesized in radiolabeled form, and their photochemical behavior in solution has been studied. The radiolabeled compounds were prepared in good yields according to improved synthetic procedures; they are stable and were obtained with specific activities in the range of 50–100 Ci per mmol and radiochemical purities in excess of 95%. A simpler model system, phenyl diazoketopropyl ether, was used to study the photochemical behavior of the diazoketopropyl ether group. Direct irradiation of this compound at 254 nm in methanol led to 33% insertion product (methoxyketone) and 67% Wolff rearrangement product (ester). Irradiation of [^{3}H]hexestrol diazoketopropyl ether (**5**) in methanol gives mainly nonpolar photoproducts (presumed to be the methoxy ketone and ester); however, irradiation in aqueous medium leads to large amounts of free hexestrol (52%). Photolysis of hexestrol azide (**8a**) in either methanol or water gives the corresponding amine in low yield as the only identifiable photoproduct.

We have attempted to take a systematic approach to the problem of developing photoaffinity labeling reagents for the uterine estrogen receptor. Our previous publications have covered in detail the rationale of our approach (Katzenellenbogen et al., 1976, 1977a) and have described the synthesis of several photosensitive derivatives of steroidal and nonsteroidal estrogens, all in nonradiolabeled form (Katzenellenbogen et al., 1973a). We have also fully described our investigations of the reversible binding affinity (in the dark) of these reagents

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