AN ISOTOPIC STUDY OF DNA-DEPENDENT RNA POLYMERASE OF E. COLI FOLLOWING T4 PHAGE INFECTION*

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SUMMARY— When either ^{14}C-glucose or a ^{14}C-amino acid hydrolysate is added to a ^{3}H-amino acid-labeled culture of E. coli B one minute after T4am82 phage infection, both ^{3}H- and ^{14}C-label are found in highly purified DNA-dependent RNA polymerase. Following phosphocellulose column chromatography, more than 95% of the ^{14}C-label associated with the enzyme is found in a component with a molecular weight of about 10,000, as analyzed by Sephadex G-200 column chromatography in the presence of sodium dodecyl sulfate. Under the conditions of these experiments, the phage infection caused no preferential loss of ^{3}H-label from the α as compared to the ß subunit, and no significant incorporation of ^{14}C into either of them.

Reports of changes in DNA-dependent RNA polymerase following T4 phage infection, particularly in the α (1, 2) and σ (3, 4) subunits, have recently appeared. Interest in these findings led to this isotopic study, designed to detect changes early in the infection process in the α, ß, and ω subunits of core RNA polymerase. E. coli B cells were labeled with a ^{3}H-amino acid hydrolysate before infection, and a part of the culture was labeled again with ^{14}C-glucose after infection with T4am82 phage. ^{14}C-glucose was used after infection so that all carbon compounds, including amino acids, would be labeled. In another experiment, a culture was labeled with ^{3}H-leucine before infection and with a ^{14}C-amino acid hydrolysate after infection. RNA polymerase was isolated from each batch of labeled cells and carrier uninfected cells by a procedure involving phosphocellulose column chromatography to remove the σ subunit. The resulting core RNA polymerases were examined for label content in the remaining subunits by Sephadex G-200 column chromatography in the presence of sodium dodecyl sulfate (SDS). The finding of ^{14}C-label in a small protein resembling the ω subunit of the normal enzyme (5) is described below. No significant isotopic changes in the α and ß subunits were detected.

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METHODS

E. coli B was grown in the low phosphate medium described by Bautz and Hall (6), containing 0.1% glucose and tryptophan (10 µg/ml), .1 mM $CaCl_2$, and 3 µM $FeCl_3$. In one experiment, a 1200-ml culture was labeled with ^3H by the addition of 4 mCi of a ^3H-amino acid hydrolysate (Schwarz Bioresearch Inc.) when the cell density had reached 2 X 10^8/ml. When the cells had reached 5 X 10^8/ml, they were collected and suspended in fresh medium containing 0.045% glucose. The culture was grown to a cell density of 6 X 10^8/ml, and T4am82 phage at a multiplicity of 10 was added to half of the culture. One minute after the addition of the phage, 2 mCi of ^{14}C-glucose (sp. act. = 240 mCi/mmole, Schwarz Bioresearch Inc.) were added. After 12 minutes, both cultures were collected by centrifugation. To each batch of cells, 50 gr of E. coli B [grown as previously described and stored frozen (7)] were added and RNA polymerase was purified as described previously (7), except that the DEAE cellulose step was omitted.

In a second labeling experiment, a 600-ml culture was labeled with 15 mCi of ^3H-leucine (sp. act. = 6 Ci/mmole, Schwarz Bioresearch Inc.) before infection, and with 1 mCi of a ^{14}C-amino acid hydrolysate (Schwarz Bioresearch Inc.) following T4am82 infection as above. The cells were collected, 20 gr of E. coli B were added, and enzyme was isolated as described above.

Each of the three labeled enzyme preparations was chromatographed on a phosphocellulose column as described by Burgess et al. (8). With both the ^3H-normal enzyme and the ^3H-, ^{14}C-glucose-infected enzyme, RNA polymerase was eluted from the column using a buffer containing 0.4 M KCl, after the column was first washed with a linear gradient (75 ml) from 0.05 M to 0.25 M KCl. In the case of the ^3H-, ^{14}C-amino acid-infected enzyme, the phosphocellulose column was eluted with a linear gradient (120 ml) from 0.05 M to 0.50 M KCl. The enzyme fractions from the phosphocellulose columns were precipitated with ammonium sulfate (0–0.6 saturated) and stored in the storage buffer of Burgess (9).

Sephadex G-200 column chromatography and SDS gel electrophoresis were carried out as described by Burgess (5).

^3H- and ^{14}C-radioactivity in the protein samples was determined by placing an aliquot of the sample on a 3MM filter paper disc, drying the sample, and counting it in toluene scintillation fluid in a Nuclear Chicago scintillation spectrometer.

Protein was determined in the Sephadex column fractions by the method of Lowry et al. (10).

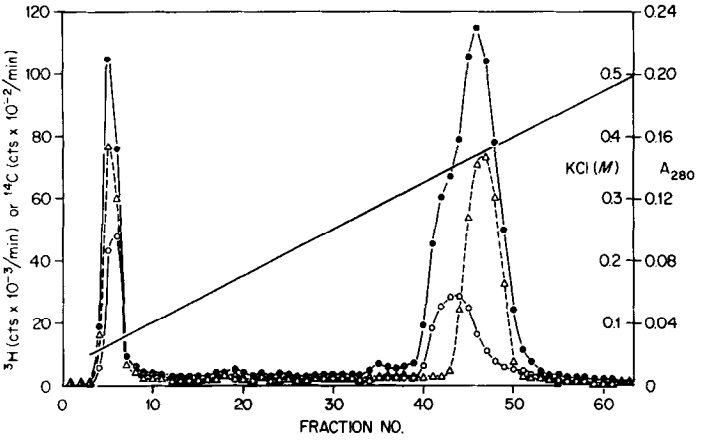

FIG. 1. Phosphocellulose column chromatography of the ^{3}H-, ^{14}C-amino acid-infected enzyme. Six mg of enzyme were dialyzed for 3 hr against 50 mM Tris buffer, pH 7.9, containing 50 mM KCl, 0.1 mM EDTA, 0.1 mM dithiothreitol, and 5% glycerol, and then applied to a 1.2 X 6-cm phosphocellulose column. After application of the sample (2.5 ml) the column was washed with 4 ml of the above buffer and then eluted as described under METHODS. Two-ml fractions were collected and aliquots of each were analyzed for radioactivity, for 280 nm absorbancy, and for RNA polymerase activity using calf thymus DNA as a template. ^{3}H-label, ●—●; ^{14}C-label O—O; A_{280}, Δ—Δ.

RESULTS

Purification of RNA polymerase from the two batches of ^{3}H-, ^{14}C-infected cells showed that ^{14}C-label was closely associated with the enzyme in both cases. Seventy percent of both the ^{3}H- and the ^{14}C-radioactivity present in the 14S enzyme peak from the first (high salt) sucrose density gradient was recovered in the 18–20S enzyme peak of the second (low salt) sucrose density gradient (7). A portion of the ^{3}H-, ^{14}C-amino acid enzyme was also chromatographed on a DEAE cellulose column following the second sucrose density gradient. The elution from the column of ^{14}C-label paralleled that of ^{3}H-label, 80% of both being associated with the peak of RNA polymerase activity.

The results of phosphocellulose column chromatography of the ^{3}H-, ^{14}C-amino acid-infected enzyme are shown in Fig. 1. Some of the ^{3}H-label (20%) and ^{14}C-label (30%) were eluted immediately from the column. This material was not further analyzed, so it is not known whether there was any label in a σ subunit. Substantial amounts of both, but particularly of ^{14}C-label (17%) were found in fractions 10–40, suggesting that there was a slow loss of material from the column. Seventy percent of the ^{3}H-label and 45% of the ^{14}C-label were eluted with about 0.35 M KCl, as is typical of core RNA polymerase (8). The broad ^{3}H peak in the region of core enzyme appears to be composed of

two ^{3}H-labeled peaks. The ^{14}C-label is associated with the first, and the absorbancy at 280 nm (of carrier uninfected enzyme) is similar to the second. Fractions 40–44 and 46–50 were combined, and each set was precipitated with ammonium sulfate and analyzed by SDS disc gel electrophoresis. The two sets of fractions both contained the α and ß bands of core polymerase and thus were similar to the two core polymerase peaks reported by Burgess (5) and discussed below. The two sets of fractions were combined for study on the Sephadex column.

The core RNA polymerases from the phosphocellulose columns were subjected to Sephadex G-200 column chromatography in the presence of SDS to analyze for isotope associated with the subunits. The chromatograms of ^{3}H-normal enzyme and ^{3}H-, ^{14}C-amino acid-infected enzyme are shown in Figs. 2A and 2B, respectively. ^{3}H-Normal enzyme shows label in the ß (first peak) and α (second peak) subunits. There is no ^{3}H-label in the region of the ω subunit. The chromatogram of the ^{3}H-, ^{14}C-amino acid-infected enzyme also shows the ^{3}H-α and ^{3}H-ß peaks. About 97% of the ^{14}C-label is eluted in the position of the ω subunit. Several known proteins were used to calibrate the Sephadex column, and from their position the ^{14}C-component was estimated to have

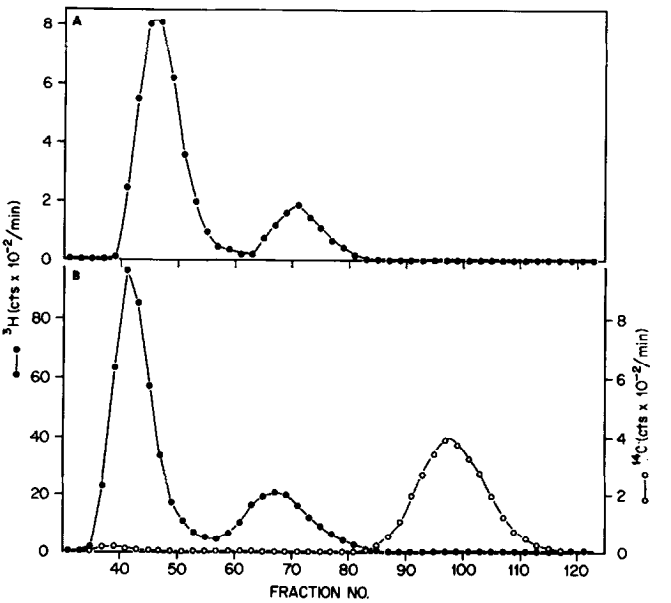

FIG. 2. Sephadex G-200 column chromatography of the labeled enzymes. Two mg of ^{3}H-normal enzyme (A) or 0.8 mg of ^{3}H-, ^{14}C-amino acid-infected enzyme (B) was dialyzed for 24 hr against 1% SDS. The sample was then applied to a 1.5 X 75-cm column (Fig. 2A) or a 1.5 X 70-cm column (Fig. 2B) of Sephadex G-200 and eluted with 1% SDS. One-ml fractions were collected, and an aliquot of each fraction was analyzed for radioactivity.

a molecular weight of about 8–10,000, similar to that estimated for the ω subunit (5). With the ^{3}H-, ^{14}C-glucose-infected enzyme, a chromatogram similar to that shown in Fig. 2B was obtained. About 2% of the ^{14}C-label was found in the ß subunit, 1% in the α subunit, and 97% in the position of the ω subunit. The ^{14}C-material eluting in the position of the ω subunit from this latter experiment was hydrolyzed with acid and subjected to amino acid analysis. Label was found with all of the amino acid peaks.

Table 1 shows the specific activities of the α and ß subunits of the ^{3}H-normal enzyme and its parallel T4-infected enzyme, the ^{3}H-, ^{14}C-glucose-enzyme. Both the α and the ß subunit of the T4-infected enzyme showed lower specific activities (26–27% less) than normal enzyme. The lower values probably are due to a lower recovery of infected enzyme during the purification procedure in the presence of the carrier normal enzyme. There was no significant difference between the two enzymes in the ratio of ß/α specific activity, both being close to 1.

TABLE 1

Specific Activities of the α and ß Subunits of the Labeled Enzymes

Fractions comprising the α and ß subunit peaks from the Sephadex G-200 columns as described in Fig. 2 were combined, and radioactivity and protein determinations were made on each.

Enzyme	α Subunit	ß Subunit	Ratio of Specific Activities
	cpm/μg	cpm/μg	ß/α
^{3}H-Normal	5.24	4.76	0.91
^{3}H-, ^{14}C-Infected	3.82	3.54	0.93

SDS disc gel electrophoresis of the core enzymes showed results similar to those obtained using the Sephadex G-200 column, although the amount of protein analyzed was much less. All of the ^{14}C-label moved ahead of the α subunit.

DISCUSSION

Following T4 phage infection, ^{14}C-label is incorporated into a small protein which is closely associated with RNA polymerase. This finding may mean that a new ω subunit is

formed after infection. The chromatographic behavior of the protein on a phosphocellulose column is similar to that described for the normal ω subunit (5). Using a gradient elution on phosphocellulose chromatography, Burgess (5) found two peaks of normal core RNA polymerase that are very similar to the two ^{3}H-labeled peaks shown in Fig. 1. He found that the only detectable difference between the two core polymerases was that the leading peak contained the ω subunit and the other did not. The present results obtained with enzyme after phage infection are similar, since the ^{14}C-protein resembling ω was found with the leading ^{3}H-core polymerase peak. However, the normal enzyme as detected by absorbancy at 280 nm showed only the second peak, and there was no evidence of a ^{3}H-normal ω subunit on Sephadex chromatography. The results suggest that the normal ω subunit may be lost during the course of the purification procedure used here, which differs in several steps from that of Burgess (5). Goff and Weber (11) have also reported that the core enzyme from T4-infected cells is eluted from phosphocellulose columns at a lower salt concentration than normal E. coli core enzyme. They did not find two peaks from infected cells, as were found in the experiment described here. The indication that an ω subunit may be labeled following T4 infection adds more significance to determining its role in the polymerase reaction. Little is known at present except that it has no apparent effect on enzymatic activity (5).

Under the conditions used in the experiments described here, no isotopic change considered significant could be detected in the α or ß subunit following infection. Seifert et al. (2) reported a change following T4 phage infection resulting in a more acidic and larger α subunit as detected by electrophoretic changes. They felt on the basis of immunological results that the α change was a modification of the old subunit rather than a synthesis of a new one. While this work was in its final stages, Goff and Weber (11) reported that, using ^{35}S-labeled E. coli cells, the ratio of ^{35}S-α label to ^{35}S-ß label in RNA polymerase did not change as a result of T4 phage infection. They reported that after T4 infection the α subunit picks up ^{32}P-label, found in 5'-AMP after isolation procedures. The studies reported here using a complete ^{3}H-amino acid hydrolysate to label E. coli polymerase support the findings of Goff and Weber (11) using ^{35}S, in that they show no preferential loss of α or ß subunit material following infection. The studies reported here with ^{14}C-glucose or ^{14}C-amino acid label after infection show that, within the time period studied, no significant incorporation of ^{14}C-radioactivity into the α or ß subunit occurs.†

†A significant incorporation of label would have been about 3–5% of the total ^{14}C-label found in the Sephadex column fractions and would correspond to a change in molecular weight of the α subunit of about 500 as calculated on the basis of the specific activity of the ^{14}C-glucose.

Similar results to those reported here have been obtained using carrier infected cells, although the overall recovery of enzyme is less. The enzyme from infected cells resembles that described by Seifert et al. (2) in its activity with T4 DNA as compared to calf thymus DNA. About 70% of the α band was found to be modified as detected by urea gel electrophoresis (2).

Further studies on the identity of the ^{14}C-protein resembling the ω subunit and its function are in progress.

REFERENCES

1. Walter, G., Seifert, W., and Zillig, W., Biochem. Biophys. Res. Commun. 30, 240 (1968).
2. Seifert, W., Qasba, P., Walter, G., Palm, P., Schachner, M., and Zillig, W., Eur. J. Biochem. 9, 319 (1969).
3. Bautz, E. K. F., and Dunn, J. J., Biochem. Biophys. Res. Commun. 34, 230 (1969).
4. Travers, A. A., Nature 223, 1107 (1969).
5. Burgess, R. R., J. Biol. Chem. 244, 6168 (1969).
6. Bautz, E. K. F., and Hall, B., Proc. Nat. Acad. Sci. U.S.A. 48, 400 (1962).
7. Stevens, A., J. Biol. Chem. 244, 425 (1969).
8. Burgess, R. R., Travers, A. A., Dunn, J. J., and Bautz, E. K. F., Nature 221, 43 (1969).
9. Burgess, R. R., J. Biol. Chem. 244, 6160 (1969).
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. 193, 265 (1951).
11. Goff, C. G., and Weber, K. K., Cold Spring Harbor Symp. Quant. Biol., in press.